

What Next for British Higher Education ?

THE nerves of British academics, newly returned from distant parts, are taut with anticipation of what Mrs Margaret Thatcher, the Secretary of State at the Department of Education and Science, will have to say about the budget for the next university quinquennium when Parliament reassembles after its own junketing at the party conferences. On the face of things, the omens are not good. At the end of June Mrs Thatcher was telling the Association of Education Committees of her belief that primary education, and especially nursery schools, must take pride of place in the education budget. Since then, word has leaked out that she plans to trim the target of 320,000 university students by 1976-77 to something like 309,000, an increase of some 6 per cent over the present university population. But the real pressure is on costs. Mrs Thatcher said in June that she would be looking for economies in the numbers of postgraduate students, the availability of two-year university courses and an attempt to persuade larger proportions of university students to live at home. In the circumstances, it is plain that this is more than ever a time for reappraising British strategy in higher education.

First of all, it must be acknowledged that a large part of the expansion foreseen by the Robbins Commission on Higher Education in the early 1960s has already taken place. The total number of students at British universities more than doubled between 1960 and 1970, and has grown still further since. During the same period successive governments have embraced the principle of a binary higher education system, and although the polytechnics are as yet making only a modest contribution to the production of graduates (a little over 10 per cent), their influence is bound to grow, and even Mrs Thatcher says as much. And it would be wrong of university academics to suppose that degree courses are the only courses which respectable students should follow—to that extent, ministerial enthusiasm for the polytechnics is justified (but there remains the suspicion that economy is misguidedly and even falsely the chief spur). Given that the output of the colleges of education now exceeds 20,000 a year, the proportion of young men and women in Britain receiving some form of formal higher education is much more respectable than it used to be.

Serious structural problems remain, however, and it is hard to think that the universities will be dealt with kindly in the years ahead if steps are not taken somehow to bridge the gaps between the three parts of the system of higher education which have now grown up. The chief difficulty is the ambiguous relationship between the universities, supported by the Central Government through the University Grants Committee, and the polytechnics, supported chiefly by the Central Government through the intermediary of the local education authorities. The constant assertion of those who defend the polytechnics is that there is a need of institutions of higher education which are in some sense more practical than the universities, yet paradoxically the universities are struggling

(partly with prompting from their students) to be more "relevant" (and inviting the wrath of Lord Robbins for their pains). In a comparatively few years the better polytechnics have established good working relationships with some universities, but are more jealous of their independence from the UGC and of their dependence on the Rate Support Grants than are the weaker brethren. In the next few years, however, the institutional separation between the two groups of institutions is likely to be confirmed, for there is every likelihood that the Local Authorities Higher Education Committee, which started functioning in July, will become the polytechnics' equivalent of the UGC with the reorganization of local government planned for 1974. So is it entirely beyond the wit of intelligent academics that some institutional means should be sought within which the two parallel systems of higher education could work in concert?

The relationship between the colleges of education and the rest of the system of higher education is still more anomalous. The report of Lord James's committee on teacher training has probably helped to confirm the Department of Education and Science in its view that teacher training is a vocational activity best conducted in isolation, yet the present system is wasteful not merely of the talents of those who might wish to become good teachers but who are unwilling to abandon academic ambitions at seventeen or so. Might it not be better that a brief teacher training should follow on one of the two-year courses on which Mrs Thatcher has set her heart than be a four-year course in what Mr Edward Short has called an "educational ghetto"? And is there not a chance that if all three types of institutions were more closely welded together, it would be easier than in the past few years to match the output of graduates to the jobs available for them, an increasing embarrassment at least for the universities?

In the circumstances it is not surprising that these must now tighten their belts, but the howls of protest there will no doubt be should above all be discriminating. The possibility of financing postgraduate students in part at least by loans, no longer actively canvassed by the government, would be a lesser evil than the sharp curtailment of postgraduate teaching now in prospect. The government's ambition to reduce the numbers of students in residence is silly, and should be resisted, perhaps most effectively, by the rejoinder that the government should do much more to encourage the building of accommodation for young unmarried adults, not merely students, up and down the country. Complaints that financial stringency are somehow a mark of lack of courage by the UGC are grossly unfair, but the universities would help themselves if they would help the committee to bring about a further rationalization of the pattern of teaching in the universities. The pressure for more two-year courses is harder to assess; the growth of the past decade has not yet been fully assimilated, and the universities have been culpably slow to understand the ways in which



the curriculum should change (which is not the same as to say that there has been no innovation) to meet the needs of students as well as those of higher education.

Dingle's Answer

EVERYBODY is fond of Professor Herbert Dingle, as well as of the clock paradox in special relativity which he has single-handedly nurtured since the early 1930s. Like Morris-dancing and the tossing of pancakes on Shrove Tuesday, the sporadic outbreaks of controversy which Professor Dingle stimulates are delightfully English in quality. For is it not marvellous that while the whole world goes about its business not merely believing in Special Relativity but using it, there should be philosophers in England willing to proclaim that Einstein was, after all, quite wrong?

The most recent development in this saga is Professor Dingle's latest book, *Science at the Crossroads* (Martin Brian and O'Keefe, £3.00). Dingle sets out not merely to restate his view that Special Relativity is false but also to document his complaint that the profession of science and the scientific journals (*Nature* prominent among them) have denied him a proper hearing. Thus he charges that *Nature* denied him an opportunity to criticize the Royal Society for not accompanying a rejection of a paper with a detailed refutation, mistaking the sympathy of one journal for another's referees for kow-towism. He recounts the history of his complaint to the Press Council in 1968 that *Nature* had suppressed the truth, and of the subsequent publication of a letter (*Nature*, 216, 119; 1968) which triggered off a further series of refutations, the most recent of which was a simple declaration by Professor J. L. Synge that "I cast my vote" for Special Relativity. Professor Dingle goes on to complain that a promised leading article rounding off the correspondence has never appeared, apparently oblivious of the way in which his own scorn for prospective contestants and his promises to "bring discredit on the journal" may have discouraged the judicious summing-up for which he asked. In his book, Professor Dingle persists however in asking for a simple refutation of his position. Will what follows do the trick?

Professor Dingle's book has the virtue of putting his question of orthodox physics in a simple form. "According to the special relativity theory . . . two similar, regularly running clocks . . . in uniform relative motion must work at different rates. Hence one clock must work steadily at a slower rate than the other. The theory, however, provides no indication of which clock that is, and the question inevitably arises: How is the slower clock distinguished? The supposition that the theory merely requires each clock to appear to work more slowly from the point of view of the other is ruled out merely by its many applications and by the fact that the theory would then be useless in practice but also by Einstein's own examples. . . ." The trouble, of course, is that in the last of these sentences, Dingle is denying the central principle of relativity. And why should he not accept that each of two clocks in uniform relative motion should appear to run slow from the other's point of view? That, according to the relativists, is what the real world is like.

The fact that one observer's view of reality may differ

from another's is not a paradox but a characteristic of the universe.

Professor Dingle's example from Einstein is the well-known prediction that a clock at the Equator would be observed to run more slowly than an identical instrument at one of the poles. But why should it not be the other way around? That is what Dingle asks. It seems now to be accepted that Einstein's original argument was uncharacteristically loose. The point of the illustration is that a clock at the pole of rotation may be taken to be in an inertial frame which is nearly (but not quite) properly defined by the direction of the Earth's motion around the Sun. The clock at the Equator is in another. Einstein's lack of clarity concerns the inertial frame of the observer of the two clocks. Dingle's difficulty is in accepting that the predictions of the special theory of relativity require great care in the definition of inertial frames and it is true, of course, that a close examination of the relationship between two such frames leads quickly to general relativity. The confusion which runs through much of the arguments which Dingle has skilfully conducted is his obstinate refusal to accept the disciplines which relativists set for themselves. Is it any wonder that he comes to contrary conclusions? And is there any hope that he will now be satisfied with the demonstration that moving clocks run at different speeds from clocks at rest which has been provided in the past few months by the experiments in which Hafele and Keating have flown caesium clocks in different directions around the world (*Science*, 177, 166; 1972, see also *Nature*, 238, 244; 1972)? It will be sad to see the clock paradox disappear, but this work is the last nail in the coffin.

100 Years Ago



NOTES

MR. DARWIN'S forthcoming work on "Expression in Man and Animals" bids fair to be of a more popular character than any of his other publications. It will commence with a statement of the general principle of Expression;—that serviceable actions become habitual in association with certain states of the mind, and are performed, whether or not of service, in each particular case. This will be illustrated in the case of expression of the various emotions in man and the lower animals. The means of expression in animals will then be discussed, and the special expressions of animals and man, such as the depression of the corners of the mouth in grief, frowning, the firm closure of the mouth to express determination, gestures of contempt, the dilatation of the pupils from terror, the causes of blushing, &c. In conclusion, the bearing of the subject will be spoken of on the specific unity of the races of man, the part will be discussed which the will and intention have played in the acquirement of various expressions, and the question of their acquisition by the progenitors of man will be referred to. Seven heliotype plates; reproduced from photographs will illustrate the work.

From *Nature*, 6, 460, October 3, 1872.

OLD WORLD

Science Research Council Advises the Government

WITH the government departments in the throes of reorganization for the coming of the customer-contractor principle, the annual report of the Science Research Council this week gives advice on how to run the system (*Science Research Council Annual Report*, Cmnd 458, HMSO £0.73).

The council insists that most of the research and development financed by the government, in particular that through the research councils, is for the general benefit of the community rather than for direct application by government departments. To ensure that industry and the professions derive full benefit from the results, the council calls on the government to ensure that the professions, universities, polytechnics, research councils, executive departments, NRDC and other government agencies "work as an integrated whole to choose national research and development programmes and to ensure the application of the results to the nation's wealth and well-being".

The council also points out that applied research and development carried out with a particular application in view is often more expensive than basic research and that, if work like this is to be properly controlled, it must have specific objectives. Until sufficient basic research has been carried out, applied research and development programmes will be premature, says the council, and will lead to "waste and duplication". The advantages of an applied research programme, however, is that gaps in knowledge emerge which can be made good with further basic research.

Having established, in principle, the need for both basic and applied research programmes, the Science Research Council goes on to say that each department "should certainly be responsible for commissioning the applied research and development which it needs . . . or for whose potential users it is manifestly the best sponsor department", but according to the report the departments should also influence the allocation of all funds for research and development programmes, including the science budget of the Department of Education and Science. In addition, the SRC calls on the departments to influence the allocation of funds between the individual councils and also to influence the research programmes of the councils.

The SRC also reiterates its warning to the government that the customer-

contractor principle will not be successful without "strong chief scientist organizations which command the confidence and respect of the scientific community".

Politics apart, the council reports that during 1971-72 it spent £59.96 million, an increase of £8.6 million over 1970-71. Of this sum £8.47 million was allocated to CERN, £4.7 million to ESRO and £415,000 to NATO scientific schemes. During the year, the SRC awarded 1,676 research grants compared with 1,501 the previous year. Engineering subjects were given priority but science subjects also showed some growth.

The SRC spent £42.66 million of its funds in universities, technical colleges or other institutions in 1971-72 with the University of Cambridge heading the list with £4.43 million. Of this the university's control engineering department was supported to the tune of £750,000, astronomy received £664,000 and mechanical and production engineering £572,000. The University of Oxford received £3.91 million with the Nuclear Physics Department receiving the lion's share of £601,000.

During 1971-72, the SRC lifted its restriction on the numbers of research assistant posts in universities except in nuclear physics. The council felt able to do this because of the apparent surplus of scientists and engineers.

The council, for the first time, is able to plan two years ahead instead of one. The proposed support for 1974-75, however, is less than the council needs—showing an increase in real terms of only 2.5 per cent over 1973-74—and the council emphasizes that the gap

between its resources and the needs of the expanding universities and polytechnics will widen. In spite of these difficulties the council has decided to increase the number of post graduate awards in 1973 by three per cent but this autumn will issue the same number as last year.

The council says that the Mark VA radio telescope, which it is hoped will be built at Meifod in mid-Wales, the Northern Hemisphere Observatory and a millimetre wavelength facility are to be given priority. The report also mentions that the Astronomy, Space and Radio Board are considering alternative research opportunities for British scientists who have been affected by the reduction in the ESRO scientific programme.

Nuclear physics, for which the SRC provided £1.91 million in 1971-72 in universities and elsewhere, will, the council hopes, be supported to the same extent in real terms in the future. The 300 GeV project at CERN will have the highest priority but the council says that it also hopes to build the nuclear structure facility at Daresbury, which has recently been shown to be a viable project (*Nature*, **237**, 192; 1972).

The company, however, believes that of its three chief areas of business—radiochemicals, radiopharmaceuticals and radiation sources—prospects of expansion are greatest in the radiopharmaceutical field, but to maintain its share of the market the report says that a flow of new products is needed. To increase its share of the market, according to the report, this must be coupled with a superior marketing and technical service.

RADIOCHEMICALS

Active Centre

IN spite of increased costs, resulting from the Radiochemical Centre Ltd being granted independent status in February 1971, the company's first annual report published last week shows a healthy profit of £816,000, thirty-five per cent more than achieved in 1970-71 (*First Annual Report*, The Radiochemical Centre Ltd, Amersham).

During the year, sales increased by 22 per cent over those made by the centre during the days when it was a part of the United Kingdom Atomic Energy Authority. An eighteen per cent return on capital was obtained, three per cent more than in 1970-71, but the return on

sales of 14 per cent is the same as obtained the previous year. The company is paying a 10 per cent dividend but it is still able to add £293,000 to its capital.

During the year, new pharmaceutical laboratories and dispensaries were commissioned at a cost of £1 million. The cyclotron which the centre uses to produce isotopes has, as well, been modified to improve its stability.

The company, in spite of its bright first year, is reluctant to make firm predictions for the future. With most of the company's products being sold abroad—£3.72 million worth last year—its income is dependent on exchange rates and, according to the report, competition is becoming stronger.

EDUCATION

Nuffield Foundation

IN spite of a decrease in income from £1.7 million in 1970 to £919,065 in 1971, the Nuffield Foundation still managed to allocate over £1 million in grants during the year ending last March. This sum is, however, £389,000 less than allocated in 1969, but the foundation had more than £1 million in reserve, on December 31, 1971.

This disastrous financial year for the foundation is the result of a much decreased final dividend paid by the British Leyland Motor Corporation in 1971. The foundation is not, however, quite so dependent on the performance of BLMC this year, for the report says that the foundation sold 14 million of its holding of 40 million shares in the company in May 1971. With BLMC having this year declared an improved final dividend as well, it looks as if the foundation in 1972 will have an income of about £1.2 million.

Grants awarded to British applicants were scarcely affected by the decreased income of the foundation, but applications from some Commonwealth countries were not so successful as in previous years. A spokesman for the foundation said last week that with the increasing sums of money available in the developed Commonwealth countries—such as Australia and New Zealand—for activities which the foundation supports, the trustees felt that they should decrease their level of support in these countries and provide more for the developing countries.

The Nuffield Foundation has done less to encourage teaching innovation in higher education than in the schools. But the report says that a grant of £95,000 was allocated last year to Professor Hudson of the Centre for Educational Research in Edinburgh University and Mr R. A. Becher of the Nuffield Foundation, to set up a group to study innovation in higher education. The programme of the group is to be divided into two parts. First the group will initiate under Professor Hudson's guidance a series of inquiries into certain significant developments in the teaching programmes of some university departments, in order to analyse and contrast the beliefs and values on which the staff base their teaching, and the expectations and assumptions of the students taking the courses. To carry out this study the group will base a research worker in each department under study, which to start with will consist of the faculty of medicine at the University of Edinburgh and the department of electronic and electrical engineering at the University of Birmingham.

Second, the group will study the overall trends in undergraduate education in Britain to find out in what sub-

jects significant changes are taking place and to investigate how the results of innovation in undergraduate teaching can be disseminated. This part of the study will be the responsibility of Mr R. A. Becher.

Other interesting projects supported include a study of the attitudes towards a career in science by the staff and students of a chemistry department within the University of London. This study, financed by a grant of £3,000, is being carried out by Dr Dorothy Zinberg, a sociologist from Harvard University who carried out a survey within the same department in 1968–69. At that time it emerged that a significant proportion of the students were disillusioned by the value of the scientific way of life and at the prospect of using their training as chemists to earn a living. Dr Zinberg will now find out whether the views of the students have hardened during three years at university.

The foundation last year also provided support for Professor Niko Tinberg, of the University of Oxford, who received £4,800 to make films illustrating behavioural research in animals. The foundation's report, however, makes it clear that this grant is an exception—"Professor Tinberg's worldwide reputation as an ethologist and as a film maker . . . proved irresistible".

DUTCH ELM DISEASE

No Immediate Relief

THERE are no signs yet that Dutch elm disease is abating in Britain. The first returns of the 1972 survey of Dutch elm disease are now being received, and although it is too early for clear conclusions to emerge the subjective opinion within the Forestry Commission is that the position is no better this year than last, and may even be worse.

The survey of 1,700 plots began six weeks ago and detailed results should be available very shortly. It appears that there have been marked improvements in certain areas over the past year, but new losses in other areas make many experts fear that still more of Britain's 18 million elms are affected, in spite of efforts made by local authorities over the past 18 months.

The only method of controlling the disease at present is by felling trees which have become infected by the fungus (*Ceratocystis ulmi*) which is carried from tree to tree by two species of elm bark beetle, *Scolytus scolytus* and *S. multistriatus*. Local authorities are responsible for control, and £250,000 has been made available by the Forestry Commission to help meet the administrative expenses, but no money is available to help with the cost of felling the trees and compen-

sating owners. The cost can be high. Up to March 1972 Croydon Corporation alone had spent £25,000 of ratepayers' money trying to eradicate the disease.

Elm disease has fluctuated over the years in Britain, reaching a peak in the 1930s, and the Forestry Commission admits that when the disease increased in 1970 they assumed—at first—that the increase was temporary. Research at Alice Holt Lodge, one of the Forestry Commission's chief research stations, was running at a low key, costing only a few hundred pounds a year. In 1971 the Forestry Commission, realizing the gravity of the situation, spent some £30,000 on research, and this year will spend £35,000, while the Natural Environment Research Council (NERC) has now started a fundamental research programme that will cost at least £23,000 a year for the next three years.

In the immediate future research offers little hope of rapid control. At Alice Holt Lodge the Forestry Commission is concentrating on the immediate problems of control, developing fungicides that can be injected into individual trees (this work is carried out in cooperation with the USA, which has been ravaged by the disease since 1930), and examining pesticidal sprays which could be used against the beetles that spread the disease. Both these methods are likely only to be of use in protecting individual trees of great amenity importance; injecting 18 million elms with fungicide is not a practical proposition.

If the disease is to be controlled in the near future it will be by destroying diseased bark. To date, £100,000 of the £250,000 that the Forestry Commission made available has been spent by local authorities, and the rest is likely to be spent this year. After that, where will the money come from? The Forestry Commission provided the £250,000 out of its income of about £16 million a year and as yet central government has refused to help local authorities with the actual costs of tree removal. As it is, some local authorities have tackled the problem zealously, but this is of little avail when a neighbouring authority, as in fact happens, takes no action.

But there are important lessons to be learnt from the present problem. Little research has been done until now on diseases of amenity trees. Now that NERC has started basic research on the population dynamics of scolytid beetles, the development of the disease, and the possibilities of using native pathogens to control the beetles—it should not be long before a fundamental knowledge of controlling diseases that attack amenity crops such as elm trees will be built up.

UNIVERSITIES

Birmingham Inquiry

THERE should be more interdisciplinary PhD degrees and PhD candidates should spend some part of their time of study in employment, according to the review body which for the past 20 months has been considering the role, constitution and functioning of the University of Birmingham.

In the report published last week (*Report of the Review Body appointed by the Council of the University of Birmingham*, University of Birmingham), Mr Jo Grimond, the chairman, and his review body produce well over 200 recommendations which range over the entire university parish from curriculum to administration. Of these, 30 or so need the approval of the Privy Council with a corresponding number needing to be approved by the university Court of Governors.

The report proposes that the faculty of science and engineering should be made into three faculties—life sciences, physical sciences and applied sciences—and that in time a faculty of environmental sciences be created. The review body also recommends that in future the council be empowered to change faculty structure and title without the permission of the Privy Council.

The review body says that a university "cannot stand aside from the moral issues and problems of society" but, as well, it must not divert from its primary duty of being an institution of higher learning. It is recommended that every undergraduate be taught at least once a week in a small class or tutorial and the review body feels that the classification of degrees should still continue and that examinations and assessments should still be part of the system at Birmingham.

The committee also recommends that the day-to-day decisions within the university be taken by an academic executive of 21 people of which 13 will be the *ex officio* members and 2 will be students. All non-academic matters and day-to-day decisions on finance and accounts should be dealt with by a 23-person Finance and General Purposes Executive committee, says the report.

The report says that the university should take all opportunities that arise to attract research prospects. In particular, it is suggested that the university tender for local authority research work and that it should join in projects involving collaboration with overseas universities. Such an approach to research, says the report, might, however, have an adverse effect on the careers of research workers if the contracts become too rigid.

Post-doctoral research staff and research students should be used more

in demonstrating and tutorial work, and departments should also be allowed to increase their proportion of research staff paid for by the university. The report says that in these circumstances departments should reorganize their teaching loads so that these extra staff will count towards the staff-student ratio.

The report also calls for greater use to be made of the facility where PhDs may be awarded to people who have spent some time in industry and who wish to return to the university to read for a research degree, foregoing residence and supervision requirements if necessary.

SOVIET SCIENCE

Emission Reactor

THE thermal emission of electrons has, for some time, been considered a promising means of obtaining a small but reliable source of electric power. At a conference on the subject held in Germany earlier this year, the possible applications of a nuclear-powered thermal emission unit in communications satellites and spacecraft was stressed. The Soviet Union has been working for some years on this problem, but in their prototype reactor, the "Romashka", the heating of the emission material was carried out through

intermediate semiconductor links, heated by the nuclear reaction.

In the new Soviet thermal-emission reactor, the "Topaz", the intermediate semiconductor stage has been eliminated. According to *Izvestiya* (September 16, 1972), the Topaz consists, essentially, of a compact uranium reactor, with a moderator of zirconium hydride. The core is "pierced by electric generator channels", arranged as a "garland" of five generating elements linked in series.

The core of each element is a small uranium cylindrical rod. The heat generated by the reactor is transmitted to the molybdenum alloy cylinder which surrounds each rod. The molybdenum acts as a cathode, emitting electrons, which are collected by a cylindrical anode some fractions of a millimetre away.

The two Topaz prototypes so far constructed have been tested for more than 1,000 h, and have been found to provide a stable electric supply of 5 to 10 kW. (The thermal output of the reactor itself is 130–150 kW.) According to Dr I. D. Morokhov, deputy chairman of the State Committee on the use of Atomic Energy, Topaz has "shown definitively that the new method of obtaining electrical power from nuclear reactors is ready to go into practical operation".

CHINA

Welcome Visitors

FOR the first time since the cultural revolution in the mid-1960s, a multi-disciplinary scientific delegation from the People's Republic of China will visit Britain at the end of next week. The seven-man delegation, which will be led by Professor Pei Shih-Chang, Director of the Biophysics Institute of the Academia Sinica, will spend two weeks in Britain visiting universities and government laboratories in London, Edinburgh, Oxford and Manchester.

The visit is the direct result of a visit made to China in May of this year by Professor Sir Alan Hodgkin, President of the Royal Society, Sir Kingsley Dunham and Sir David Martin. It is hoped that next week's visit will pave the way for a resumption of regular visits of Chinese scientists to Britain which existed until the mid-1960s. At that time some 30 or so Chinese scientists, who were generally at the post-doctoral level, used to visit Britain each year for anything up to six months.

As well as Professor Pei the delegation will consist of Professor Pai Chieu-Fu who is a member of the presidium of the Scientific and Technical Association of the People's Republic of China and a leading member of the Peking

Municipal Bureau of Science and Technology; Professor Chang Wen-Yu, a high energy physicist, who is deputy director of the Institute of Atomic Energy of the Academia Sinica; Professor Chien Wei-Chang of Tsinghua University, whose field is dynamics; Professor Chien Jen-Yuan a high polymer chemist, who is director of the Chinese Chemical Society and a fellow of the Chemical Institute of the Academia Sinica; Mr Hu Shih-Chuan, a scientist at the Shanghai Institute of Biochemistry; and Mr Li Fu-Sheng, deputy director of the Shenyang Laboratory of the Computing Science and Technological Institute.

The visit to London seems to mark a concerted plan for forming closer links between China and the outside world. In the past few weeks, the National Academy of Sciences in the United States has also been surprised (and pleased) to find that its long-standing invitation to the Academia Sinica to send a delegation to Washington has been taken up, and that a delegation of Chinese scientists will be in Moscow in the second half of October. Its visit will overlap with that of a Russian delegation, also due in time for the academy's autumn meeting. It will be interesting to see whether the American academy chooses to throw the two delegations together.

NEW WORLD

Delaney Amendment Once More in Question

by our Washington Correspondent

ALLEGATIONS were flying around on Capitol Hill last week that the Food and Drug Administration (FDA) is not properly regulating some food additives, that a committee of the National Academy of Sciences is biased in favour of industrial concerns, that the food industry fraudulently deceives consumers with flavourings and colouring agents and that the basis of the law governing the regulation of food additives in the United States is a scientific anachronism. The setting was Senator George McGovern's Select Committee on Nutrition and Human Needs, which held three days of hearings on the safety and need for food additives in the United States.

The hearings were an important landmark in the regulation of the quality and safety of American food for they brought into the open, in no uncertain manner, many of the arguments surrounding the workings of the food and drug laws and in particular the so-called Delaney Amendment, which attempts to prevent carcinogens from entering the food supply. The hearings also took place against the background of moves in Congress to take the FDA out of the Department of Health, Education and Welfare, and place it in a new Consumer Protection Agency. And they also adequately reflected the growing concern among consumers about the safety of processed foods—a concern which is evidenced by the burgeoning market in so-called health foods.

The hearings brought out familiar arguments about the safety of some specific additives, such as monosodium glutamate, nitrites and diethylstilbestrol, but they also saw a stream of criticisms directed against the Food Protection Committee of the National Academy of Sciences, which advises the FDA on the safety of many food additives. Another recurring theme in the hearings was the question of whether or not all the 3,000 additives now licensed for use are really needed. The food industry was accused of deceiving consumers by making foods appear what they are not.

But perhaps the most important and far-reaching aspects of the discussions concerned the Delaney Amendment and in particular the suggestion that it should be weakened and made more flexible. Linked to this discussion was an exhaustive consideration of two bills sponsored by Senator Gaylord Nelson, who chaired the committee while

Senator McGovern was on the campaign trail. The bills are designed to extend the Delaney Amendment to cover teratogens and mutagens as well as carcinogens, and to improve the testing of food additives before they reach the market.

Enacted in 1958, the Delaney Amendment has been a source of controversy ever since. It is direct in intent and rigid in interpretation, for it simply states that no chemical found to cause cancer when fed to animals can be used as a food additive. The basis of the amendment came from a meeting of the International Union Against Cancer in 1954, which drew a distinction between reversible and irreversible effects of chemicals on man, and which agreed that no threshold levels below which there is no threat to health can be defined. That contention is the reason why the Delaney Amendment prescribes a total ban on potential carcinogens, and allows no tolerance levels to be set.

But the contention is under fire on several fronts, and the FDA is sitting in the middle, uncomfortably caught in the crossfire. As Charles C. Edwards, Administrator of the Food and Drug Administration, makes no secret of the fact that he would like to see some changes in the Delaney Amendment, a position which he shared last week with Lloyd W. Hazleton, founder of a toxicology laboratory, who testified on behalf of the Grocery Manufacturers' Association. Dr Hazleton argued that the rigidity of the Delaney clause precludes the use of scientific judgment in determining whether or not a chemical is safe in the food supply, and he pointed to the familiar arguments that it is impossible to extrapolate effects of chemicals on laboratory animals to the situation in man. Few scientists, he suggested, hold the view that only a molecule or two of a carcinogen is sufficient to cause cancer, and that it is therefore scientifically unjustified not to allow tolerance levels to be set for potential carcinogens.

Dr Edwards, although careful not to criticize openly the Delaney Amendment, which he is forced by law to uphold, told the committee of the many headaches that it causes for the FDA. One of his chief arguments is that since 1958 there have been great strides in analytical techniques, so that it is now possible to detect the presence of minute quantities of additives in the food supply. He suggested that with this

sensitivity in analytical techniques, the concept of zero tolerance, if taken to its logical extreme, would require "banning of all animal and plant products containing not only detectable levels of naturally occurring carcinogens . . . but also all food containing such carcinogenic environmental contaminants as traces of radioactive material".

When asked directly by Senator Nelson whether he would like to see the Delaney Amendment abandoned, however, Dr Edwards said that he would not, but added "I think the Congress as well as the scientific community has got to take a good hard look at Delaney, not with the idea of doing away with it, but bringing it into the 1972s scientific world, if you will, but there needs to be some kind of Delaney. My personal feeling is . . . that we need some increased flexibility in interpretation of scientific results". Senator Nelson was quick to point out that if flexibility means the authority to set tolerance levels, that would effectively mean abandoning the philosophy of the Delaney Amendment.

Dr Samuel Epstein, a pathologist from Case Western Reserve, who has long been a supporter of the Delaney Amendment and a critic of present food additive regulations, immediately took issue with Dr Edwards's statements. To allow the setting of tolerance levels for potential carcinogens, he said, "would open the floodgates" for the incorporation into the food supply of chemicals whose effects are at best uncertain. He also characterized Dr Edwards's suggestion that the Delaney Amendment, if taken to its logical extreme, would lead to the banning of foods with naturally occurring carcinogens and radionuclides, as a red herring. Dr Epstein pointed out that most legal interpretations of the amendment are that it applies only to intentional additives.

As for the arguments that most chemicals are carcinogenic when tested at high doses, Dr Epstein told the committee that such statements "reveal a total lack of comprehension of the gross insensitivity of toxicological procedures". Testing at high doses is necessary, he said, to overcome the insensitivity imposed on animal tests by the small sample sizes routinely used—an effect at low doses sufficient to induce cancer in one animal per ten thousand would require an experimental group of at least 30,000 rodents. Moreover, Dr Epstein pointed out that the concept of zero tolerance which underlies the

Delaney Amendment was "unanimously and unequivocally endorsed" by an ad-hoc committee of recognized authorities in the field of chemical carcinogenesis, which was appointed by the Secretary of Health, Education and Welfare in 1969, after the amendment was responsible for removing cyclamates from the market.

Apart from the Delaney Amendment, another fundamental issue discussed at the hearings was the suggestion, embodied in one of Senator Nelson's bills, that chemicals found to produce mutagenic or teratogenic effects in animals should also be totally banned from the food supply. Dr Edwards argued against such an extension of the Delaney Amendment, chiefly on the grounds that teratogenesis in animal tests has been reported as a result of injecting substantial apparently as harmless as common salt and zinc, or feeding a diet of raisins or excess vitamin A. He also argued that experiments with chick embryos, which are extremely sensitive to teratogens, would rule out the addition to the food supply of such essential substances as calcium and one of calcium pantothenate (an essential B vitamin).

But Dr Epstein offered his enthusiastic support for the proposed extension of the Delaney Amendment to include teratogens and mutagens, pointing out that the effects of such agents can be distinguished from other toxicity effects by their irreversibility. As for Dr Edwards's arguments about injecting possible teratogens into animals or hens eggs, Dr Epstein pointed out that the Delaney Amendment requires appropriate testing, and that such tests are not appropriate for the evaluation of the safety of a food additive—an appropriate test, he said, would be a feeding study.

Several participants in the hearings were also concerned about the burgeoning growth of the food additive market—now estimated to be worth about \$500 million a year—and the deceptive practices of the food industry in adding flavourings and colouring to disguise the absence of other ingredients, or to create a false impression that a product is packed with nutrient. Ms Anita Johnson, an Attorney connected with a Washington based consumer group called the Health Research Group, called such practices fraudulent, particularly when the presence of additives is not clearly advertised on packaging. But Dr William Lijinsky, a biochemist from Oak Ridge National Laboratory, summed up the feelings of many of the witnesses last week when he expressed concern about the number of additives on the market, and especially about the fact that few have been tested for synergistic effects. "It is hard to approve the use of the American public in a large scale chronic toxicity study", he said.

NUCLEAR POWER

All at Sea

by our Washington Correspondent

THE Westinghouse Electric Corporation believes it has a neat answer to environmentalists' criticisms of nuclear power plants—operate the plants at sea. Not long ago, the concept of a floating offshore power plant was considered to be a pipe dream, but it came a step nearer reality last week when Offshore Power Systems, a company formed jointly by Westinghouse and Tenneco, the largest shipbuilding concern in the United States, signed a contract with a New Jersey utility company for two floating nuclear power stations. The plants will be moored side by side about three miles off the New Jersey coast in about 45 feet of water, and if all goes according to plan, they should be providing electricity by 1980. But the project still has some troubled waters to navigate, even before the first construction rigs are put into place.

The idea is to construct the plants in a new assembly yard being built in Jacksonville, Florida, and then to tow them to their moorings, fully completed. They will be surrounded by a massive semicircular breakwater designed to provide protection from even the most severe storms and from stray ocean-going liners. Construction is expected to start on the breakwaters in 1976 and the plants will be towed into place three years later. Each plant is designed to generate 1,150 MW.

Westinghouse is quick to point out all the possible environmental advantages in siting a power plant at sea. Not only will such a plant take the pressure off riverfront development, thereby leaving more land for "recreational purposes", but it will also be possible to dissipate the cooling water and radioactive discharges easily in the sea. The company's spokesmen even claim that the slight temperature increase in the water around the plant will be beneficial for fisheries in the area and may also open up the possibility of setting up fish farms.

As for cost, the total outlay on the enterprise is estimated at \$1,100 million, far greater than for conventional plants. Of this total, \$750 million will go towards construction of the plants, \$100 million for transmission lines and \$250 million for the breakwater. It seems a high price for Westinghouse to pay for protecting the environment. But the company is looking on the venture as the key to a whole new market for power stations.

For one thing, New Jersey is rapidly running out of riverfront property suitable for power plant construction, and so a new approach has to be tried. But, more important, Westinghouse and Tenneco are setting up the construction

yard in Florida not just for these two plants but for a whole series of carbon copies ready for sale to any utility that may want them. In the past, power plants have been individually designed to meet criteria dictated by the construction site, but offshore power plants can all be built to the same design. By standardizing construction in a conveyor belt operation, Westinghouse and Tenneco hope eventually to cut down on costs by eliminating time overruns, site investment and, since construction time will be shorter than for conventional plants, borrowing costs will be lowered. Moreover, since all the plants produced in Florida will be built to the same design, the companies hope that the lengthy business of licensing hearings will have to be gone through for only the first one or two plants.

But the future for the project is not as rosy as the companies like to suggest. For one thing, three bills have already been introduced into the New Jersey legislature to block construction of the plants. Then jurisdictional disputes about the ownership of offshore areas are likely to arise, which may lead to licensing delays. Finally, not everybody is convinced about the environmental advantages of siting plants at sea. In particular, doubts have been raised about the ability of the breakwater to withstand the full force of a tidal wave or an earthquake and anxiety has arisen over the navigation hazards associated with the plant. All these doubts and fears will have a chance to be aired when the New Jersey state government holds hearings, probably next year, and when the AEC holds its licensing hearings a year later.

Astronomical Coordination

An ad-hoc meeting of 40 astronomers from 27 institutions in North America met at the University of Michigan last month to discuss coordination of observations of variability in extra-galactic objects. The aims of the meeting were to identify important experiments involving the monitoring of extragalactic objects, to discuss ways of coordinating such experiments and to look at the feasibility of establishing a data bank for their results. One committee was set up to promote regular exchanges of observing plans between experimenters and cooperation between institutions, and another was set up to look at the feasibility of a data bank to which observers of extragalactic objects would contribute data in a standard format when their work is accepted for publication. The bank would be accessible to all astronomers. The committee to assist in planning and cooperation needs suggestions: its chairman is Mark Stull, Radio Astronomy Institute, Stanford, California.

More Coals of Fire for Club of Rome

THE World Bank (International Bank for Reconstruction and Development) has now joined the lists of commentators on the Club of Rome's first encyclical (*The Limits to Growth* by Professor Dennis Meadows and his colleagues at the Massachusetts Institute of Technology) with a slashing attack by a task force drawn from its staff and consultants. The full text of the task force's report will be published later in the year as one of the bank's occasional papers. The chief criticisms of the *Limits to Growth* centre on the way in which this computer model deals with population, non-renewable resources and pollution, but there are also serious criticisms of the nature of the model itself. The task force says that its study has been hampered by the lack of detailed knowledge of the background and the assumptions on which Professor Meadows's computer model is based.

The task force, which says it has done its best to avoid the rhetoric which has attended the publication of *The Limits to Growth*, begins by saying that a "model is just as good as the assumptions which are built into it". It says that it has found that "many of the assumptions in the model were not scientifically established and the use of data was often careless and casual". The report also says that "contrary to the protestations of the authors", the model is fairly sensitive to the assumptions which are made and that "reasonable adjustments in the assumptions regarding population, non-renewable resources and pollution could postpone the prediction of catastrophe by another 100 or 200 years even if one accepted the general methodology of the model".

Population

The World Bank report is less critical of the way in which the model deals with population than of the other assumptions on which it is based. Acknowledging that the model is right to postulate that population has been growing exponentially for a century and that the assumptions about population in the medium run are "fairly sound", the task force goes on, however, to say that the model "does not do justice to a number of factors which are likely to come into play in the long run and which may even be significant in the short run". It points out that fertility is already declining in several countries—no fewer than 56 of the 66 countries for which accurate data are available at the United Nations already show a decline of fertility. It complains that the model links fertility and

mortality predominantly with economic factors, and that it makes inadequate allowance for voluntary restraint of population. The task force acknowledges that population growth is a serious matter, but says that the real issue is how to arrest population growth by voluntary means and by the use of more efficient contraceptive techniques suitable for poor nations.

The task force takes a cautious view of the prospect that demographic stability will be brought about in many now-developing countries by the demographic transition which has characterized Western Europe in the past century. It says that although mortality and fertility in a country have in the past been closely associated with the stage of economic development in a country, "in recent years, these relations are becoming weaker", and that the association between prosperity and reductions of fertility as well as of mortality may "further weaken" in the years ahead. The source of this opinion seems to be the lack of correlation between reduced mortality and economic development in the poorer nations since the Second World War.

But there is some hope in the deliberate use of family planning programmes. Indeed, the task force says, there is evidence that the intensity of a family planning programme is positively correlated with the degree to which fertility will decline "independently of social and economic forces". The task force points out that the funds needed to finance family planning programmes are so modest that there is no reason to fear that governments will have to wait for substantial economic growth to be able to afford them—none of the assumptions in the Meadows model—and it goes on to say that evidence now available from India, North Africa and Latin America as well as China suggests that the peak of the growth rate of the world's population will be reached much earlier than in the most recent projections of the United Nations.

Non-renewable Resources

The World Bank's task force is more fierce in what it has to say about the treatment of non-renewable resources in *The Limits to Growth*. It says that several of the assumptions in the model are "extremely pessimistic". One of its more telling points is that several of the figures for known reserves of metal ores and other natural commodities are taken from statistics published by the US Bureau of Mines, but that "the small print has been ignored . . . which

warns that 85 per cent of the reserve estimates have a confidence level of less than 65 per cent". The report says that the authors of *The Limits to Growth* have adopted these estimates "indiscriminately" and have apparently been unaware that other supposedly authoritative studies of commodity reserves cited in their work are also based on the same data, similarly used.

Another complaint is that the authors of the computer model have extrapolated estimates of demand by the US Bureau of Mines for the year 2000 and have extrapolated them into the future in circumstances when it must be doubtful whether they can be used "so heavily and dogmatically". One of the sources of bias to which the task force draws attention is the understandable preoccupation of the US Bureau of Mines with ensuring security of supply to the United States. The result is that the bureau does not pursue an exhaustive investigation of resources elsewhere in the world once it is assured that the United States will not be short of essential materials. Contingency forecasting of this kind is said to be entirely appropriate in the circumstances for which it is intended, but unsuitable for making global projections.

The task force also complains at the use of estimates of supply of raw materials fixed as they are at present and estimates of demand which increase exponentially into the future. This, the report says, sets a scenario in which "the only trouble-free growth rate would be zero" and this, "of course, is absurd". The report says that no allowance is made, in the discussion of non-renewable resources in *The Limits to Growth*, of the way in which growth curves can level-off in S-shaped fashion, no allowance for the extent to which some nations may avoid the steep parts of the growth curve by the rational use of resources and no allowance for the ways in which the pattern of use of raw materials may change. It also points to the margins of error associated with exponential projection into the future. Lowering the projected growth rate of a mineral from 4 per cent a year to 1.5 per cent a year is, the report says, equivalent to lengthening its life fivefold. "Exponentiality is, after all, a double-edged sword." The predictions of disaster which spring from the assumptions in the Meadows study about non-renewable resources spring from the assumption of "irrationality".

The task force goes on to paint a more positive picture of the future of non-renewable resources. First, it says

that the small size of the known reserves of some important materials stems in part from the way in which those who look for minerals have little incentive to identify reserves that will not be needed for more than 30 years or so. Second, it points to the way in which the known reserves of important metal ores have often in the recent past increased more quickly than the rate at which they are consumed. Known reserves of iron ore, now the equivalent of 250,000 million tons of iron, have increased five-fold since 1954, and it is reckoned that a price increase of less than 40 per cent would allow the economically usable ores to be doubled again. But the Meadows study used an estimate only half as great as the present known reserves usable at present prices. The present known reserves of copper are 3.5 times greater than those known in 1935, and a three-fold price increase would multiply them by 2.5. Bauxite reserves have multiplied seven times in the past 22 years. Both lead and tin are now less scarce than they were thought to be in 1929.

The task force does not deny to threat of scarcity for some materials, but says that present discussions are characterized by "uncertainty rather than finiteness". And when the authors of *The Limits to Growth* claim to have "corrected for the whole basket of uncertainty" by multiplying proved reserves five times, they have made a claim which is "patently absurd" by ignoring the extent to which known reserves may be still further increased by technical developments yet to come, especially in geophysical exploration.

The report also list some of the facts "ignored" by the Meadows study, first rejecting the claims for the MIT model that its elegance is a better guide to the future than history as "grossly erroneous". It complains that the computer model ignores the way in which technology can increase available reserves, as in the extraction of nitrogen from liquid air and sulphur from salt domes, the extraction of important quantities of metals from previously dumped material, the possibility of extracting aluminium from clay and oil from municipal garbage and the resources of the seabed. The World Bank estimates that it should be possible to recover 100 million tons a year of manganese nodules from the sea within one or two decades, thus increasing by factors of between three and twelve the present reserves of manganese, nickel and cobalt at production costs only a fraction of current prices.

Pollution

The task force says that "the assumptions regarding pollution are the weakest part of the model as in many cases they are not established on any scientific

basis". It complains that in the model, the output of pollution is determined by the growth in capital stock alone and says that if the ratio of pollution to capital stock were reduced to three-eighths of the value assumed, well within the range of error, "the prediction of catastrophe would be completely erased". It goes on to say that there is "absolutely no scientific evidence" for the assumption that the capacity of the world to absorb pollution is merely four times the amount of pollution now produced.

The task force also says that the evidence for the assumption in the computer model for a link between mortality and pollution is non-existent.

Acknowledging that pollution is an important issue in many industrialized countries the task force nevertheless says the assumptions regarding pollution in the computer model "are ill-considered", that the model does not fully allow for the way in which advanced societies can choose to spend extra money on the control of pollution and that the model ignores the comparative cheapness of removing large amounts of pollution—it quotes estimates that it would be possible for the United States to achieve a substantial reduction in pollution in the next six years at a cost of \$16,000 million, one-third of the expected increase of the Gross National Product in that time. One of the kindest sentences in the report is that "This does not mean there is no danger; it does mean that pollution build-up and world collapse is not necessarily inevitable even with continued economic growth."

The Model Itself

Hitherto, most criticisms of *The Limits to Growth* have centred on the assumptions and have tended to avoid criticism of the model itself. The World Bank is less discreet. It says that neither the original Forrester nor the Meadows version of it is "really a model, nor is it really about the world". It may have helped to get people interested in the subject, but at this stage the question is not how to save mankind but how to improve the model.

One technical objection to the study is that it does not investigate projections beyond the year 2100, which means that the nature of the predicted instabilities is not defined on questions such as carrying capacity of the Earth, the task force says that it would be more reasonable to assume that capacity increases with time. It goes on to say that the assumptions made about parameters such as the reserves of non-renewable resources undermine the calculation of carrying capacity which the model attempts.

There is also a fierce criticism of the use of exponentiality in the model, for

in an exponential model "things either explode or die out". But this, the task force says, is not the real world, where observed relationships are much more intricate. It complains that no economist would follow the computer model in applying exponential growth to a gap between supply and demand.

The task force criticizes the way in which the work of Professor Meadows and his team seeks to identify direct relationships between pairs of variables and then goes on to use these as if they were natural laws when the variables lie well outside the observed ranges. The result, the task force says, is that with the assumption explosive exponentiality, there is an unwarrantable amplification of one variable by another.

The task force says that, in spite of appearances, the MIT model "is really quite small". Half of the 121 equations are merely auxiliaries irrelevant to the functioning of the model itself. The report complains that these relationships are a hangover from the Dynamo model and that "those who have been trained in Dynamo seem to write equations this way out of habit even though the system no longer requires such rigid behaviour on their part".

The most serious criticism of the model as such is that some of the equations on which it is based are such as to force some of the more spectacular results of *The Limits to Growth*. Thus the great growth of industrial capital, defined on an old-fashioned economic theory as output less consumption, agricultural investment and investment in services, includes depreciation in such a way as to include the effects of an increase of the average life time of capital on the one side of the equations with the result that "the model behaves perversely . . . this is an example of the type of misleading underspecification that runs all through the system".

In the same vein, the task force complains that in the relationships assumed between industrial production, industrial output, agricultural output and the output of the service industries, the parameters are chosen in such a way that the model must always predict collapse. And one of the most curious parts of the model is the treatment of technology and the assumption that even in "an age of the most dramatic technological progress", there will in future be relatively little change.

The task force thinks that the chief danger now is that people will be drawn into fighting some of the false issues raised by the MIT study rather than into more constructive fields. The World Bank says that it will not, as it might have done, pursue a further exploration of the model on its own account—that is a task for others to take up.

NEWS AND VIEWS

Precursors of Immunoglobulin

THE function of the heterogeneous RNA of high molecular weight (HnRNA) that is synthesized by the nucleus of eukaryotic cells has long been the topic of speculation. This RNA, characterized by its rapid turnover and broad sedimentation pattern in sucrose gradients (20–100S), is largely retained and degraded in the nucleus with no more than about 20 per cent reaching the cytoplasm in the form of an RNA of lower molecular weight. On the basis of labelling kinetics and hybridization studies it has been proposed theoretically that HnRNA is a precursor of mRNA, a postulate which is not difficult to accept as the large and small ribosomal RNAs of eukaryotes originate from a precursor molecule approximately twice the required size. The kinetic and hybridization studies have, however, not been definitive, the latter because of the redundant sequences present in the genome of eukaryotes. But viral sequences have been shown to occur in the HnRNA of viral infected cells.

Two rather direct experimental approaches are available which should solve the puzzle. One involves the synthesis of the complementary nucleotide sequence of a purified mRNA by one of the viral RNA-dependent DNA polymerases, a feat already accomplished with the haemoglobin mRNA, and hybridizing it with HnRNA. If the mRNA sequences are present in the HnRNA the anti-mRNA should hybridize with it. It is, of course, critical that the mRNA be pure and that it be copied faithfully. This general approach has been used by Melli and Pemberton (*Nature New Biology*, **236**, 172; 1972) for examining the relationship between haemoglobin mRNA and the HnRNA of duck erythroblasts. The other approach is to assay directly the HnRNA for mRNA activity in a heterologous system, looking of course for the synthesis of a specific protein. This approach assumes that the non-mRNA sequences will not interfere with the template activity of the HnRNA, and it requires a sensitive assay because of the difficulty of obtaining large quantities of HnRNA and because the mRNA may represent a small portion of the precursor sequences. The assay developed by Gurdon, Lane and Marbaix involving microinjection of mRNAs into *Xenopus* eggs and oocytes provides the required sensitivity (as little as 1 pg of the haemoglobin mRNA can be assayed), and the first reported use of eggs to detect the mRNA activity of HnRNA was described by Stevens and Williamson in *Nature* recently (**239**, 143; 1972). They isolated the RNA containing polyadenosine from the nuclei and cytoplasm of cultured myeloma cells, synthesizing IgG immunoglobulin by differential extraction and retention on poly(U) columns; the hope was that the immunoglobulin mRNA as well as its precursor(s) would contain a sequence rich in adenosine and therefore lend itself to this simple isolation procedure.

When the cytoplasmic and nuclear RNAs prepared by this method were injected into *Xenopus* eggs and allowed to incubate for four hours in the presence of radioactive amino-acids the synthesis of immunoglobulin (H_2L_2)

could be detected by antibody precipitation and by polyacrylamide gel electrophoresis of the antibody precipitate. These results demonstrate that the light and heavy chain immunoglobulin mRNAs do indeed contain a sequence rich in adenosine, an observation also made by Swan, Aviv and Leder (*Proc. US Nat. Acad. Sci.*, **69**, 1967; 1972) for the myeloma light chain mRNA. More important, however, these results are consistent with the argument that HnRNA contains the immunoglobulin mRNA sequences. The critical question, which the authors are certainly aware of, is whether the nuclear RNA is contaminated with cytoplasmic RNA—a possibility rendered unlikely because no 18S ribosomal RNA could be detected in the nuclear preparation—or whether the nucleus contains a small pool of immunoglobulin mRNA either synthesized directly or already processed from a precursor RNA. This is particularly critical as 40 per cent of the nuclear RNA migrates faster than 28S ribosomal RNA in gels, and is therefore smaller.

It is further shown by Stevens and Williamson that heavy and light chains are assembled into a 7S myeloma protein (H_2L_2) by *Xenopus* eggs, a process in which the vesicles of microsomes have been implicated (Vassalli, Lisowska, Bernstein and Lamm, *J. Mol. Biol.*, **56**, 1; 1971). Therefore eggs must contain some polysomes bound to the membrane which have not yet been observed, or some other membraneous material must serve this function. It is possible that only the microsomal disulphide interchange enzyme is required for assembly and that it is present in eggs.

The synthesis of assembled immunoglobulin by *Xenopus* eggs is even more surprising in light of the recent findings by Milstein, Brownlee, Harrison and Mathews (*Nature New Biology*, **239**, 117; 1972) who have shown that the light chain of mouse myeloma is synthesized in the form of a precursor molecule. When light chain mRNA isolated from MOPC 21 mouse myeloma is added to a Krebs II ascites cell-free system, authentic light chain is synthesized (*FEBS Lett.*, **23**, 244; 1972). When the same RNA is added to a reticulocyte cell-free system, however, light chain synthesis is not observed but a new protein that migrates more slowly appears in the polyacrylamide gels. The slower protein is also observed when myeloma polysomes are incubated with Krebs II ascites supernatant factors while the microsomes from which the polysomes are derived make authentic light chains. The precursor nature of the slower migrating protein is further supported by peptide analysis. The light chain of MOPC 21, IgG, has three tryptic peptides containing methionine and one—possibly two—of these is found in the putative precursor while the other, the basic N-terminal tryptic peptide, is absent and a new basic peptide is observed. This suggests that the putative precursor contains a modified N-terminus and, from the estimates of molecular weight, this could be as many as fifteen amino-acids. It is not known whether the C-terminus is also modified. The electrophoretic mobility

of the precursor does not seem to be due to carbohydrate, for neuraminidase failed to alter its migration and it is unlikely that the reticulocyte lysate would contain the specific polysaccharide machinery for synthesizing. It therefore seems that light chains are made in the form of a slightly larger precursor, and that the vesicles of microsomes are required for converting this precursor to its final form. Reticulocyte lysates contain only free polysomes while ascites cell extracts probably contain

some membraneous material thus allowing for the conversion of the precursor to light chains.

The authors further speculate that the short amino-acid sequence at the N-terminus of the precursor may be the signal for membrane attachment of polysomes, an event which occurs after chain initiation has begun, and therefore may play a part in the secretory process. The precursors of mRNA and proteins will be the subject of much work in the next few years.—From a Correspondent.

Moon-making in Three Dimensions

ALMOST every theory of the formation of the planetary system requires the existence of a "solar nebula" in the broadest sense of some diffuse material in the vicinity of the Sun at some early stage in its evolution. Most theories regard the material as having originally about the same relative abundances of the elements as in the Sun. They generally regard it as occupying about the region of the present system in the sense that the material had to be in this region for anything relevant to happen to it. Otherwise theories differ enormously in the picture of the solar nebula, treating it as a regular distribution of material having some simple motion of circulation about the Sun, or as forming a pattern of large vortices, or as highly turbulent material, or as part of a more extensive cloud with which it continues to exchange material and momentum, or as material controlled by a magnetic field, and so on.

In his article on the origin of the Moon on page 263 of this issue of *Nature*, Don L. Anderson uses the essentially simplest picture of the initial state of the nebula, but it is well to remember that even as a starting point it is only one of the array of possibilities just mentioned. On the adopted picture, cosmogonists discuss the distribution of temperature and pressure and thence infer the extent to which elements and compounds (metals, carbon, silicates, ices, and so on) condense out of the gaseous phase to form small solid grains, or to which gaseous components may be driven out of the nebula. Thus they derive for the relevant epoch a chemical composition depending primarily upon distance from the Sun. Each planet is then supposed to "accumulate" from the material of the nebula in an appropriate zone, the different compositions of the planets being supposed to depend primarily upon the differences between the zones, although time-dependences of some of the process may also have an effect.

Coming to satellites, theories differ greatly depending upon whether they emphasize similarities or differences between the planetary system and a satellite system. Again, theories of the origin of the Moon differ according to whether they emphasize its similarity to the other principal satellites regarding absolute dimensions, or its difference from them regarding dimensions relative to those of the associated planet (the ratio of the mass of the Earth to that of the Moon being almost ten times greater than for any other such pair). Anyhow, at present interest is mostly focused upon the Earth-Moon system simply because empirical knowledge about it is incomparably greater than about any like system. Also the Apollo missions have almost given the illusion that as

much is now known about the Moon as about the Earth.

One obvious problem presented by the pair is that of their exceedingly different composition, which places the "accumulation" theory in a serious dilemma. For if Earth and Moon accumulated in the same zone, we should expect them to have similar compositions. On the other hand, if the Moon accumulated somewhere else in the system and was then captured by the Earth, it would be an example of a body whose composition is not characteristic of the zone where we now find it; but this almost contradicts the case for explaining the composition of bodies by a zoning of materials.

Anderson finds a way out of this dilemma effectively by going into an extra dimension. He points out that the calculation of the composition of the solar nebula would give a dependence upon distance from its median plane as well as distance from the Sun. Whereas the Earth may be supposed to have accumulated close to the plane, if the Moon formed by accumulation it would necessarily have drawn its material from a much larger range of distance on either side of the plane. Anderson obviously finds that the pursuit of this idea and its testing taxes all the manifold resources of the relevant chemistry and mineralogy, but he evidently draws encouragement from the results, so far as they go.

Anderson's ideas are in a general way similar to those of A. E. Ringwood (*J. Geophys. Res.*, **75**, 6453; 1970), which have been developed by other authors as well. Ringwood takes account of the time-sequence of various processes, as does Anderson, but Anderson's idea of also taking account of the variation of composition away from the median plane is apparently new in this context.

The fission theory (in any of its forms) and the capture theory of lunar origin have met with such severe criticisms that it has become a challenge to the "accumulation" theory to show that it can do better. Anderson's work and the like certainly shows that it meets with no obvious contradiction. But it then becomes so complicated that it is hard to see how any crucial test could be proposed within the frame of the work itself. Hopes of some decision seem to lie in two directions—first, the mechanics of the process: it requires to be shown that the accumulation process would actually operate and that it would be expected to produce one Moon with the correct mass, spin momentum, distance from the Earth and so on; second, comparative studies: it requires to be shown whether the processes proposed would account for the Galilean satellites of Jupiter, Titan (Saturn) and Triton (Neptune), all of which have mass and size very similar to those of the Moon.—W. H. McC.

PROTEINS

Ends and Enzymes

from our Molecular Biology Correspondent

THE evolution of solid state techniques has transformed the craft of peptide synthesis into a relatively routine, or at least not too intimidating, operation. This development has opened the way to a variety of diverting stratagems, such as elimination or replacement of individual side chains in enzymes to see what happens to the structure and activity. The state of the art is not yet, of course, such as to encourage any lighthearted attempts at synthesizing whole enzymes. What is needed, rather, is an enzyme from which an interesting oligopeptide can be reversibly detached after specific cleavage of the chain at a single point. One can then shuffle the permutations of composition, sequence and length of synthetic analogues of the oligopeptide. This strategy has been pursued to great effect by Anfinsen and his colleagues on staphylococcal nuclease, which can be assembled non-covalently from various fragments, and by Scoffone and Hoffmann and their co-workers on the pancreatic ribonuclease S-protein-S-peptide system. The S-peptide comes from the N-terminus, and is prepared by exposure of the enzyme to subtilisin. It has recently been found that under the action of pepsin a C-terminal peptide can be split off the other end of the chain, again with reversible loss of enzymatic activity.

Lin *et al.* (*J. Biol. Chem.*, **247**, 4763; 1972) have now explored the effects of changes in size and composition of the terminal fragment, and of the length of the residual ribonuclease chain, on binding and reactivation of the catalytic activity. Pepsin breaks the chain between residues 120 and 121, so that the last four residues of the native enzyme are eliminated. Carboxypeptidase can then be used to nibble away one or two further residues from the end, the second of which is the active site constituent, his-119. The truncated enzyme is inactive, and can be reactivated by addition of overlapping peptides of different lengths. The first curious observation is that C-terminal peptides containing fewer than nine amino-acids are capable of inducing only traces of catalytic activity. When the nonapeptide (116-124) is added to the core (1-118), the activity jumps to 60 per cent of native, and as the length of the peptide is increased so the activity rises still further, reaching 98 per cent for the complex of 1-118 with 111-124. The same pattern (though with slightly lower activities) emerged when the residual chain was one or two residues longer. The activities, it should be noted, do not depend on the strength of

the protein-peptide association, for they refer to saturation conditions with a large molar excess of peptide. The association constant can be independently evaluated by measuring the activity as a function of mole ratio of peptide to protein, and was found also to rise with increasing length of the peptide. Evidently then the overlapping residues in the peptide interact in an energetically desirable manner with the protein.

Lin *et al.* have, moreover, hit on a nice method of establishing whether it is the side chains of the protein or the peptide that participate in the globular conformation, in particular which of them contributes the active centre his-119 side chain. The criterion is that, in the native state, his-119 has greatly enhanced reactivity towards alkylating agents. Ribonuclease 1-120, for example, which has very little enzymatic activity, also has low reactivity at this residue. The complexes were accordingly carboxymethylated with iodoacetic acid, and the extent of carboxymethylation in the protein and peptide determined. With complexes involving ribonuclease 1-120, the protein histidine reacts preferentially by a factor of four, whereas with 1-119 the ratio is 1:1. Thus, as in a similar complex of staphylococcal nuclease fragments, there are two active configurations of comparable energy.

In a further study, Lin *et al.* (*ibid.*, 4768) have examined the importance of the residue phe-120, which lies close to

the substrate pyrimidine ring, and, as judged by n.m.r. experiments, seems to interact with it. Peptides with fourteen residues were synthesized, in which the phenylalanine was variously replaced. With leucine or isoleucine in its place, the enzymatic activity and the association constant of peptide and protein were both reduced, but only by one order of magnitude. This level of activity is correlated with the reactivity of his-119. When the phenylalanine was replaced by tryptophan, activity was down by two orders of magnitude. Evidently then there is no enzymatically important interaction of the phenyl ring with the pyrimidine of the substrate. Instead it must be presumed to be implicated in a hydrophobic cluster, which stabilizes this part of the chain, and probably keeps his-119 properly oriented in the active centre. The indole is evidently too big to fit into the slot without distortion. A particularly striking feature, which lends support to this general picture, is that in spite of the lower activity of the leucine and isoleucine analogues, the Michaelis constants for hydrolysis of cyclic CMP, and also the binding constant for the inhibitor 2'-CMP, are indistinguishable from values for the enzyme containing phenylalanine. The authors suggest that the impaired activity of the analogues may result from imprecisions in the geometry of the active site.

A similar approach has been adopted by Katsoyannis and Zalut (*Biochemistry*, **11**, 3065; 1972) to determine

Hybrid Cells and DNA Repair

WHAT should prove to be a very useful new approach to the problem of elucidating the biochemistry and genetics of DNA repair in mammalian cells is described in next Wednesday's *Nature New Biology* by Goldstein and Lin. They have exploited the fact that fibroblasts from patients with the autosomal recessive genetic disease xeroderma pigmentosum are unable to repair their DNA after it has been damaged by ultraviolet irradiation, and are therefore easily killed by such irradiation to select hybrid cells one parent of which is a xeroderma pigmentosum fibroblast and the other a golden hamster cell lacking the enzyme hypoxanthine-guanine phosphoribosyltransferase.

The karyotypes of these hybrid cells are very variable and change as the cells are repeatedly subcultured. After about forty-five generations twelve cells from one particular clone were examined in detail; the chromosome numbers of these cells ranged from 110 to 160 of which eight to sixteen chromosomes were identified as being from the human parental cell. Analysis of the glucose-6-phosphate dehydrogenase iso-

zymes in cells of this and other clones of hybrids revealed both the human and the hamster molecules as well as a hybrid molecule.

Goldstein and Lin, having satisfied themselves that the cells which survived their selection procedure were indeed hybrids, then measured the ability of the hybrid cells to survive ultraviolet irradiation and to repair damaged DNA. About 50 per cent of hybrid cells and hamster parental cells survived doses of ultraviolet irradiation that allowed less than 10^{-4} of xeroderma pigmentosum cells to survive. Furthermore direct comparison of DNA repair activity after irradiation indicates that the hybrid cells have a repair activity intermediate between that of the two sorts of parental cells.

These data indicate that hamster repair enzymes can repair human DNA damaged by ultraviolet irradiation, and that they can partially compensate for the specific defect in xeroderma pigmentosum cells, namely excision repair. Obviously further exploitation of appropriate hybrid cells may help to piece together the biochemistry of this process.

which parts of the insulin molecule are inseparable from hormonal function. It is known that the N-terminal end of the B-chain and the C-terminal end of the A-chain are not essential for activity. In spite of interactions observed crystallographically between the N-terminal ends of the two chains, the last three residues of the B-chain can in fact be removed with little consequence in terms of activity. The new work shows that generation of insulin from intact B-chains and a synthetic A-chain, lacking the last four residues at the N-terminus, is quite inactive. Evidently therefore this part of the molecule is important for biological activity.

LASERS

Tuning Revolution

from a Correspondent

RESEARCH into tunable lasers has made striking progress in the past two years, and the current development of such coherent sources became clear during a conference organized by the Institute of Physics and held at Heriot-Watt University, Edinburgh, between September 6 and 8. The conference revealed that tunable coherent sources could be found for almost all parts of the electromagnetic spectrum from 0.25 μm to 300 μm .

The conference took the form of a series of invited and contributed papers covering techniques applicable in different spectral regions, and included dye lasers, non-linear optical techniques such as optical parametric oscillators and difference frequency mixing, semiconductor diode lasers and the spin-flip Raman laser. Dr B. B. Snavely (Eastman Kodak, Rochester, New York) began the conference with an account of progress in the development of continuous wave dye lasers. These can now provide tunable radiation of about 1 W between 0.55 μm and 0.7 μm , and he foresaw extensions to the near ultraviolet and infrared regions up to 1 μm . Pulsed dye lasers, discussed by Dr E. G. Arthurs (Queen's University, Belfast), provide considerably higher powers, in the megawatt range, in pulses of duration between 10^{-12} s and 10^{-6} s. The range covered with one dye is typically 50 to 100 nm, but by utilizing different dyes and pumping lasers a total range from 0.25 μm to 1.2 μm may be achieved. The dye laser also seems likely to be capable of obtaining increasingly shorter pulse durations, because of the inherently broad-band nature of the laser transition which could provide, in principle, pulse durations of less than 10^{-13} s, although the results currently achieved are around a few picoseconds (10^{-12} s).

The region of the spectrum which generated most interest and discussion

was that extending from 1 μm to 25 μm . It is in this region that most molecules exhibit their vibration-rotation "fingerprint" — indeed, usually three times over—and therefore the impact of tunable coherent sources, with their million-fold improvement over conventional sources and spectrometers in spectral brightness (power per unit bandwidth) and resolution, becomes most significant. Already, the entire range is covered at low powers (milliwatts continuous wave) with semiconductor diode lasers, although, as was made clear by Dr E. D. Hinkley (Massachusetts Institute of Technology), a large number of separate diodes are required to fill in the whole range. Parametric oscillators and new non-linear materials were discussed by Dr D. C. Hanna and Dr R. C. Smith (Southampton University) who showed that high powers, of the order of a few kilowatts, with pulsed operation were available over a considerable tuning range, 1 to 4 μm and 8 to 12 μm . Recent experiments at Southampton have indicated that, by combining parametric oscillation with difference frequency mixing, continuous tuning from 2 μm to 20 μm should be possible.

By far the highest powers and narrowest linewidths currently available in this region are provided by the output from the spin-flip Raman (SFR) laser. In his paper Professor S. D. Smith (Heriot-Watt University) emphasized the versatility of this device, which at present may be tuned from 5 to 6 μm and from 9 to 14 μm . Over the first of these ranges, continuous wave operation with linewidths down to 1 kHz (less than 10^{-7} cm^{-1}) have been measured, and in the latter range peak pulsed powers of around 1 kW have already been achieved, together with a linewidth of a few hundredths of a wavenumber. Extensions to cover the entire far infrared are current possibilities.

In an invited paper covering applications of the spin-flip Raman (SFR) laser to gaseous pollution monitoring, Dr L. B. Kreuzer (Bell Telephone

Laboratories, New Jersey) discussed his use of the SFR laser with a spectrophotometer to detect trace quantities of gases (such as NO, C_2H_2 , SO_2 , and so on) down to concentrations smaller than one part in 10^9 . Outstanding improvements in sensitivity and selectivity were reported. The impact of the narrow linewidths on quantitative estimation of one constituent of a mixture without separation was most impressive. As most gases possess characteristic absorption spectra even in a restricted region of, say, 5 to 6 μm , development work on the SFR laser could well result in a superior alternative to the conventional mass spectrometer. Dr Kreuzer discussed the use of the laser in combination with a gas chromatograph for high resolution, short time measurements of the resulting fragments.

The conference closed with a panel discussion led by the conference secretary, Dr M. J. Colles (Heriot-Watt University), which summarized the three days of proceedings and presented conference participants with a unified picture of the techniques now available throughout the spectrum. Having established the present state of the art, the panel was invited to speculate on what might happen in the next two years. The opinions indicated that tunable coherent sources would provide complete coverage from 0.2 μm to 1,000 μm and that a revolution in spectroscopic methods seemed inevitable.

SOLID STATE

New Ways with Electrons

from a Correspondent

A NEW method of mapping the Fermi velocities of electrons in a metal, involving the measurements of microwave power, has been devised by Falk *et al.* (*Phys. Rev. B.*, **6**, 377; 1972).

Most experiments designed to study the behaviour of electrons in metals yield information in the form of some average over a large number of electrons. Although the electrons that par-

Understanding Aromatic Cage Molecules

THE bonding in the planar unsaturated sulphur-nitrogen species (S_3N_2 , S_4N_3^+ and S_5N_4^+) can be rationalized in terms of the simple Hückel theory, for there are $4n+2$ π -electrons, just as in aromatic hydrocarbons. In the related unsaturated cage molecules, typified by S_4N_4 and S_8^{2+} , the nature of the delocalized bonding is, however, much less clear, and the problem is complicated by the presence of interatomic distances intermediate between normal single bond lengths and van der Waals separations.

In a communication in next Monday's

Nature Physical Science, Banister shows that the observed geometry of these molecules can be interpreted in terms of a flexible σ -bonded framework in which sulphur atoms lie at some or all of the corners of an approximately regular polyhedron. After subtracting σ -bonding electrons and electron lone pairs, it is found that each polyhedron gives the same number of higher energy bonding orbitals as electron pairs remaining for delocalized bonding. This approach may well improve the understanding of these apparently illogical aromatic cage molecules.

icipate will generally have energies very close to the Fermi energy, it is seldom possible to disentangle such averages in order to find out the properties of a particular group, for example the speed of electrons travelling in a particular direction in the crystal (the Fermi velocity). A notable exception to this, however, is an experiment in which the microwave surface impedance of a metal slab is measured in a magnetic field of low intensity. This technique, known as magnetic surface state resonance, has been used by Koch and his colleagues (*Phys. Rev. B*, **5**, 3866; 1972) to achieve a point by point mapping of the Fermi velocity in copper.

Now Falk *et al.* have come up with a technique somewhat similar to surface state resonance. Microwave power is transmitted across a metal slab by electrons moving in slightly curved trajectories in a magnetic field and intersecting one or sometimes both surfaces of the slab. By alerting the intensity of the field, the curvature of the electron trajectory is changed and there is, in consequence, a variation in the power transmitted through the slab. From a series of such experiments a map of Fermi velocities can be built up, but, by contrast with magnetic surface state resonance, very few other data about the behaviour of electrons in the metal are needed.

This kind of detailed knowledge about the electronic motions in metals is of value both in the elucidation of other phenomena and also in a closer examination of different theoretical approaches. Such experiments do, however, place heavy demands upon the purity and structural perfection of the metal crystals, and a wider application of the methods must await appropriate developments in this area.

THIN FILMS

New Light on Epitaxy

from our Materials Science Correspondent

A RECENT paper dealing with epitaxy concerns a process which the authors, Metois, Gauch, Masson and Kern, quaintly christen "post-nucleation"; what they mean by this is that epitaxy can be "nucleated" after actual crystal nuclei have already been formed on the substrate (*Thin Solid Films*, **11**, 205; 1972).

Only one thing can be said with complete conviction about the phenomenon of epitaxy: it is not simple. Remarkable and sometimes contradictory observations abound; for instance, Russian work described in these columns last year (*Nature*, **223**, 524; 1971) suggested, in disagreement with some earlier findings, that electric fields emanating from a substrate can influence the orientation of evaporated metal films. The

central questions about epitaxy are these: what physical forces constrain a newly deposited crystal to align itself in a particular orientation relative to a monocrystalline substrate, and by what mechanism do these forces express themselves? Why just that orientation and no other, for a particular combination of deposit and substrate? Why does the orientation often change as experimental variables (including the nature of the crystal species, temperature and deposition rate) are changed?

If the past few years have seen considerable progress in answering at any rate some of these questions, much of the credit is due to the remarkable work done by Masson, Kern and their collaborators at the Université de Provence in Marseilles. Their most recent publication maintains the high standards of elegance, directness and clarity that one has come to expect.

The investigators cleaved KCl substrates in ultrahigh vacuum, immediately vapour deposited aluminium or gold on the (100) cleavage face, heat treated the film *in situ*, and characterized the deposits by electron diffraction (to determine orientation) and electron microscopy (to determine the size distribution of crystallites). By comparing size distributions before and after heat treatments, and by examining the migration of crystallites beyond the original

sharply defined confines of the film, the investigators were able to monitor the exact behaviour of the crystallites, which were extremely small (typically some tens of Å in diameter).

For each deposited metal, at low temperature, a [111] fibre texture was formed: this happened because the crystallites originally deposited (presumably) in fully epitaxial orientation, and then migrated and underwent "Brownian rotation", while all the time maintaining (111)_{metal} parallel to (100)_{KCl}. The distribution of crystallite remained invariant. At higher temperatures, crystallites impinged on each other during their migrations and merged—but there was no classical "Ostwald ripening". By some as yet unexplained mechanism, the crystallites also rotated in the process of merging, so that for many grains (100)_{metal} was then parallel to (100)_{KCl} and also $\langle 0\bar{1}1 \rangle_{\text{metal}}$ was parallel to $\langle 0\bar{1}1 \rangle_{\text{KCl}}$ —that is, there was full epitaxy, not a fibre texture. At intermediate temperatures, both forms of epitaxy coexisted, and selected-beam electron microscopy showed that the unmerged small crystallites retained the (111) texture while the larger crystallites acquired (100) epitaxy. All this indicates that both forms of epitaxy were instituted after the crystallites were already in existence—hence the term post-nucleation. The authors

Organization of Chromatin and Chromosomes

How basic histones and acidic nuclear proteins associate with DNA in the chromatin of eukaryotic cells so as to allow the regulation of gene expression is a problem that has tantalized cell and molecular biologists for years. And the subject, about which contradictory data and conflicting speculations abound, remains a matter of considerable controversy. For example, in *Nature New Biology* next week, Paul and More report a series of measurements of the template activity, the ratios of DNA to protein and the proportion of free DNA phosphate groups present in native calf thymus chromatin and in chromatins reconstituted *in vitro* from DNA and either total histones and acidic proteins, or total histones or fractionated histones. They also describe the structures of these various nucleoprotein aggregates as revealed in the electron microscope.

To cut a long story short Paul and More interpret their data as showing that, although about 50 per cent of the DNA phosphate groups in chromatin are free to react with the basic dye toluidine blue, few if any stretches of DNA that are large enough to constitute transcriptional units exist free of associated protein. Last year, also in *Nature New Biology* (**229**, 101; 1971), Clark and Felsenfeld reached precisely

the opposite conclusion from data obtained by different techniques—at present it is a case of paying your money and taking your pick. Both groups perforce used difficult experimental techniques and obviously both cannot be correct in their interpretation, but from the data available so far it is not clear which group is in error.

A not unrelated question, namely how different genes are organized with respect to each other in eukaryotes, also receives an airing from Cox in next week's *Nature New Biology*. Last year Ohta and Kimura (*Nature*, **233**, 118; 1971) argued from calculations of the mutation rates of proteins in mammals that a large proportion of the genome cannot be essential for survival. They, of course, assumed that in a single cell the mutation rates at different loci are similar, but Cox now suggests that mutation rates at different loci in the same cell may differ considerably and that these different rates are genetically controlled. Making this assumption, he argues that genes in eukaryotes may be organized not according to their function but into different segments of the genome which have different intrinsic mutation rates. In other words Cox postulates that slowly evolving genes will be physically segregated from rapidly evolving genes.

comment pointedly that it is not reasonable to seek a single theory of epitaxial nucleation.

These elegant experiments still cast no light on why (111) epitaxy is preferred at low temperatures and (100) at high temperatures, and how and why the crystallites rotate to a new epitaxy as they merge by combination of smaller colliding crystallites. Nevertheless, this work takes understanding of epitaxy a good way forward.

PRIMATOLOGY

Disappointing for Some

from a Correspondent

THE Oregon Regional Primate Research Centre acted as host to the Fourth International Congress of Primatology, held in Portland, Oregon, between August 15 and 18. The congress programme was divided into innumerable sessions of short contributed papers, and four principal symposia, covering the fields of primate behaviour, craniofacial biology, reproductive behaviour and pathology. Although very little new material was presented at these symposia, they did give non-specialists the opportunity to familiarize themselves with the results of recent research and to assess the trend of current research in these fields.

The symposium on primate behaviour fulfilled these objectives particularly well, even if the quality and coherence of some contributions left much to be desired. Three vital issues were raised: first, the limitations of an outlook centred on higher primates; second, the value of field experiments; and third, the extent of intraspecific variation in behaviour and social organization. In recent years, primate field studies have become increasingly rigorous in both concept and methodology, as might be expected in a discipline chiefly pioneered by anthropologists and psychologists with little formal training in field biology, and too often showing scant awareness of the extensive literature on the behaviour and ecology of other animals. (Until recently even the prosimian primates had been largely overlooked.)

It was refreshing, therefore, to hear Dr J. F. Eisenberg (Smithsonian Institution) consider primate societies in the broader context of mammalian social systems and expose the fallacy that higher primates are somehow superior to other mammals. He argued that examination of the behavioural repertoires, communication signals and learned traditions of selected mammalian species did not support the theory that higher primates necessarily enjoy a more complex social organization. The importance of field experiments has been ably demonstrated by

Dr H. Kummer (University of Zurich) in his earlier work on hamadryas baboons, and its value was re-emphasized by his latest report. Observations on harem groups of hamadryas baboons suggested that a social inhibition prevented more powerful males from attacking weaker males and appropriating their females. A series of experiments with captured animals was designed to test this hypothesis, and his findings confirmed the existence of a social inhibition. Too little attention has previously been paid to variations in the social structure and behaviour of a species. It was therefore instructive to hear the contribution of Dr J. S. Gartlan (University of Bristol) on intra-specific variations in the behaviour of African cercopithecines. His results clearly showed that the group organization of a single species can vary according to its ecology and that, for instance, patas monkeys, who form one-male groups in East Africa, can be found in multi-male heterosexual groups in West Africa.

The symposium on craniofacial biology produced two outstanding studies on dental variability in living primates. The paper presented by Dr D. L. Greene (University of Colorado) was specially interesting because of its application to the study of fossil hominids, and particularly to showing whether the Australopithecines represent a single sexually dimorphic taxon or two different species. His research on dental metric variation in gorillas, chimpanzees and humans has revealed

that sexual dimorphism was considerably greater in the gorilla. A comparative statistical analysis of mean differences in the male and female dentition of these three groups with mean differences in the dentition of the robust and gracile Australopithecines indicated, however, that the differences in the fossil forms could not be explained by sexual dimorphism. Dr D. G. Steele (University of Alberta) presented an elegant analysis of variation in forty-three non-metrical traits in the Tupaiidae. Among other things he showed that the dentition of *Ptilocercus* is markedly different from the rest of the Tupaiidae, and that the genus *Tupaia* can be divided, on the basis of these traits, into two distinct groups. Although this technique has proved extremely useful in studies of functional anatomy and population genetics, Dr Steele was careful to warn participants that it has only a limited value for taxonomy.

In recent years Dr R. P. Michael (Institute of Psychiatry, London) and his colleagues have produced some outstanding research on the hormonal mechanisms controlling sexual and aggressive behaviour in macaques. It was interesting therefore to hear the contributions of two former associates of his in the symposium on reproductive behaviour. Dr J. Herbert (University of Cambridge) presented a lucid review of recent work by himself and his co-workers on receptivity and attractiveness in rhesus macaques and talapoin monkeys. Earlier studies had

Excitation-Contraction Uncoupling in Muscle

IN fast striated muscle fibres, a system of radial invaginations of the outer membrane (the T system) ensures the inward propagation of the excitatory signal. This structure is an essential link in the excitation-contraction coupling. A rather selective disruption of the T system can be achieved by an osmotic shock: the fibre is soaked in a Ringer solution made hyperosmotic with glycerol, and then returned to normal Ringer solution afterwards. This procedure "uncouples" the fibre; action potentials are still generated and propagated but they do not produce a contractile response any more (Eisenberg and Gage, *Science*, **158**, 1700; 1967, and Howell and Jenden, *Fedn. Proc.*, **26**, 553; 1967).

In next Wednesday's *Nature New Biology* Zachar, Zacharova and Adrian report experiments concerned with the mechanism of this uncoupling, in which simultaneous records of electrical and mechanical activities have been made throughout the various steps of the osmotic shock procedure. Soaking the fibre in the glycerol-Ringer solution

hardly affected its resting or action potentials, but mechanical tension was transiently abolished before recovering rapidly to a normal (or even supra-normal) level. The return into normal Ringer solution first produced spontaneous contraction waves but eventually abolished the mechanical response, while membrane potentials remained practically unchanged. Later, the resting potential became less negative, and the early after-potential disappeared. Moreover, vesiculation made the fibre cloudy.

By exposing the fibre again to the first glycerol-Ringer solution the changes of membrane potentials and vesiculation were readily abolished. On the other hand, the recovery of tension depended on the conditions used in the first step of the procedure: complete reversal of the uncoupling could only be obtained if the glycerol concentration was less than 150 mM, and the soaking did not exceed 45 min.

The results are new data about the osmotic shock as a tool for investigating the excitation-contraction coupling.

suggested that oestrogens primarily determined receptive behaviour in female macaques, but Dr Herbert now believes that oestrogens are chiefly responsible for stimulating male sexual interest whereas androgens control the female receptivity. His sophisticated experimental techniques certainly provided convincing evidence of this, although some participants expressed minor reservations about his interpretations of the results. Dr G. S. Saayman (Port Elizabeth Museum, South Africa) showed how similar techniques could be applied to the study of free-living chacma baboons. His findings supported the conclusions of previous laboratory experiments.

One of the most stimulating contributions of the entire congress was presented by Dr B. A. Lapin (Sukhumi Primate Centre, USSR) in the symposium on pathology. His paper reviewed recent research undertaken in his laboratory on the possible transmission of a human leukaemia virus to monkeys. These experiments established that monkeys treated with human leukaemia material developed leukaemia and that this could then be passed on to other monkeys. Although there seems little doubt that leukaemia was transmitted virally from one monkey to another, there is as yet no direct evidence that a human virus was responsible, for a latent virus in the monkey could have been activated by the introduction of human leukaemic material.

SELENOLOGY

More Luna 20 Analyses

from our Soviet Correspondent

DETAILED analyses of the specimens of lunar rock collected by the Luna 20 probe are now revealing more than the element abundances previously reported (see *Nature*, **237**, 256; 1972). The Luna 20 specimens were collected from the eastern side of a slope (gradient 8 to 10°) in a typical highland area near the Apollonius C crater. In the immediate vicinity of the collection site there were only a few shallow craters of diameter 0.5 to 1.0 m and occasional rocks of diameter not more than 1 to 3 cm. The specimens were collected using a special hard-bit drilling and coring device. The mean diameter of the particles was 70 to 80 μm , although there were more particles of diameter more than 1 mm than in the Luna 16 (Mare Fecunditatis) specimens. There were far fewer fused specimens than in the Luna 16 sample. Experiments in ultraviolet, visible, and infrared light gave a much higher value for the albedo than for the maria specimens (Luna 16 and Apollos 11 and 12) and the colour of the Luna 20 specimens (light grey) was much lighter. Infrared spectroscopy showed the Luna 20

samples to have a more crystalline structure than that of the maria specimens; in general there was a close resemblance to the spectrum of terrestrial anorthite An_{100} , although there were some differences in the contours of the bands.

Perhaps the most interesting feature of the Luna 20 specimens, however, is their iron content. The sample collected consists predominantly of anorthosite-type rocks, in which metallic iron inclusions of various forms and dimensions are found. These inclusions occur, apparently, more frequently than in maria specimens, although Academician A. P. Vinogradov, writing in *Priroda* (No. 8, 1972), stresses that more reliable statistics would be required before a definite assertion could be made. The metallic iron particles from Luna 20 differ, however, from those of the Luna 16 specimens in that the latter contained more than 10 per cent nickel, whereas those from Luna 20 had only a negligible nickel content or no nickel content at all. Almost 50 per cent of the metallic iron from Luna 20 is finely dispersed. It does not suffer atmospheric oxidation at ordinary temperatures, and this, says Academician Vinogradov, is an "extremely remarkable fact". An experimental model for the formation of this dispersed iron has been worked out,

based on the reduction of divalent iron to metallic iron during the heating of basalt to 1,500° C *in vacuo*. Iron dispersions produced in this way are also not subject to atmospheric oxidation.

In general, the nickel content of the Luna 20 material is high, particularly in the crystalline fragments, a feature which agrees with the data of the Apollo 14 specimens (Fra Mauro Highlands) but differs from the maria samples. The lack of nickel, therefore, in the metallic iron particles of Luna 20 is one of the "unsolved questions" posed.

Another interesting problem is that thrown up by studies of fossil particle tracks. Examination of olivine from the Luna 16 and Luna 20 specimens showed that the latter had a considerably lower track density ($\rho \lesssim (1 \text{ to } 3) \times 10^4 \text{ tracks cm}^{-2}$) than those of the Luna 16 regolith ($\rho \lesssim 7 \times 10^7 \text{ tracks cm}^{-2}$). This indicates that the Luna 20 olivine, and hence the entire rock specimen, has been exposed to cosmic radiation on the Moon for a relatively short time. As the highland rocks are considerably older than the maria in current selenological theories, this result is again puzzling. Considerable further study of the process of formation of anorthosites, both in terrestrial and lunar conditions, will clearly be necessary.

Is Cen X-3 a Neutron Star?

THE X-ray "pulsar" Centaurus X-3 has a period of 4.8 s, greater than the period of any known radio pulsar. This has led to suggestions that Cen X-3 might be a pulsating white dwarf—as pulsars were thought to be before the discovery of the Crab Pulsar with its period of 33 ms. But E. P. J. van den Heuvel and J. Heise, of Utrecht, believe that Cen X-3 can be explained in terms of neutron star models, and in next Monday's *Nature Physical Science* they develop details of some such models.

Because Cen X-3 is an eclipsing binary, there is much more information available about the system than about most pulsars. From simple geometry and orbital data provided by the eclipses (which occur at 2.087 day intervals and last for 0.488 day) van den Heuvel and Heise determine an upper limit of $0.7 M_{\odot}$ for the mass of the "pulsar" component of the system. This upper limit is consistent both with white dwarf models and with neutron star models; if Cen X-3 alone is considered, there seems little *prima facie* reason to choose either sort of model. But there are other X-ray "pulsars" with periods shorter than that of Cen X-3, and if it is assumed that they belong to the same family, neutron star models become more plausible, unless overtone pulsations of white dwarf stars are invoked to explain the phenomenon.

Van den Heuvel and Heise assume that Cen X-3 does indeed contain a neutron star and go on to consider the possible evolution of such a binary system which could result in the presence of an active, yet slowly spinning, neutron star today. They suggest that, as the two stars originally forming the system have evolved, mass has been transferred back and forth. According to this model the more massive star evolved more rapidly at first, resulting in expansion and the transfer of mass to the secondary, then, about 1.7×10^6 years after this first stage of mass exchange, the original primary exploded as a supernova, leaving a neutron star remnant which became the new secondary. So the original secondary has become the primary (more massive) component, and has now evolved to the stage where mass is being transferred back to the neutron star remnant of the original primary. In such a model it is this mass exchange which has activated the neutron star, producing X-radiation as matter falls along magnetic field lines onto the poles of the neutron star, which is assumed to be rotating. The long period of the pulsar compared with other pulsars is then explained because the neutron star is old and has had time to slow down considerably since its formation as a rapidly spinning supernova remnant.

The Structure of Liquid Water

M. C. R. SYMONS

Department of Chemistry, University of Leicester, Leicester LE1 7RH

Professor Symons describes the outcome of work at Leicester on water structure.

THE topic of the structure of liquid water is frequently presented as being very obscure, difficult, and clouded in mystery. Certainly many conflicting and often widely differing theories have been propounded, and certainly many physical properties of liquid water are peculiar when gauged against those of more "normal" fluids. I contend however, that water has the structure, or rather, range of structures, that would naturally be expected in terms of the known electronic structure of the monomer (which is, of course, quite normal), and its known chemical properties which are also unexceptional. In view of the emerging possibility of checking these ideas against computer models^{1,2} it seems worth while to present a brief description of their salient features.

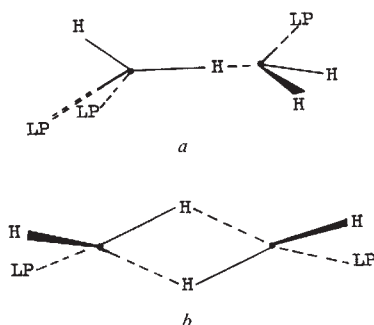


Fig. 1 *a*, Linear dimer formed by two water molecules; *b*, possible cyclic dimer postulated to exist in carbon tetrachloride solvent⁵.

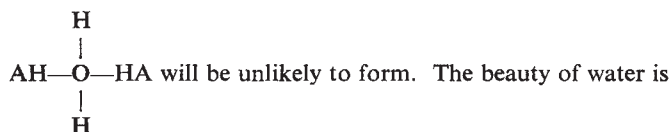
Most liquid water models that have been described are designed to reproduce selected properties of liquid water, such as its temperature of maximum density or its X-ray scattering pattern. This seems to be rather unfruitful, for almost all such models can be contrived to fit these facts. I conclude that these properties are likely to be explicable in terms of most reasonable theories for water and so confine myself to being as chemically and physically reasonable as possible.

Excellent quantum mechanical calculations for the monomer and dimer are now available^{3,4} and can be trusted to give a fair description of their structures. It seems that the conventional view of the molecule as a near-tetrahedral configuration involving two hydrogen atoms and two nonbonding electron pairs is reasonable, and that the preferred dimer structure is likely to be linear as in Fig. 1*a*. (Linear dimers have indeed been detected in solid nitrogen matrixes, but recently it has been suggested that dimers in dilute carbon tetrachloride solution are cyclic as in Fig. 1*b* rather than linear⁵. My own view is that the latter species are in fact linear but undergoing a rapid interconversion that averages certain lines in the spectrum). In the various crystalline ices, water molecules are strongly hydrogen bonded to four neighbours, and most theories for the liquid are based upon the idea that much of

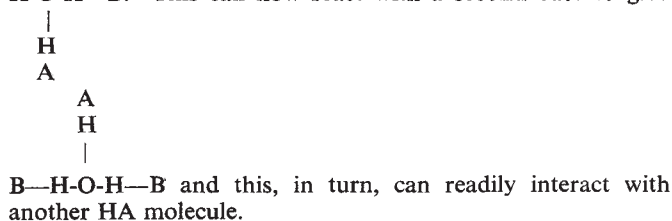
this structure is retained. As monomeric molecules appear in the gas phase it is reasonable to suppose that some monomers may be present in the liquid, and certainly that some molecules may be hydrogen bonded to less than four neighbours.

I cannot give a detailed description of the many current models for liquid water here, but most have been adequately described by Eisenberg and Kauzmann⁶. They range from extremes in which ice-like structures coexist with monomeric molecules to structures in which two or more ice structures are in rapid equilibrium. The accent is almost always upon ice.

Before returning to the early ideas of water as a polymer, I must stress a simple chemical property. When a dibasic acid, H_2A reacts, the resulting anion HA^- is a weaker acid than H_2A by many orders of magnitude. So also, but to a smaller extent, where water forms a single H-bond to a base, B, the resulting unit, $HOH-B$, is a far weaker H-bond donor. Hence, unless B is, relatively, a strong base, the species $B-HOH-B$ will not be formed. Similarly as water is amphoteric and as there are two "lone pairs" of electrons capable of H-bonding, the unit H_2O-HA will be far less basic than H_2O itself, and the species



its double bifunctional acid-base behaviour coupled with the near-tetrahedral directional property. Thus the capability of the proton as a H-bond donor in $H-O-H-B$ is largely restored if the oxygen is simultaneously involved as a base, as in $H-O-H-B$. This can now react with a second base to give



Bearing this in mind, one can visualize a process in which the concentration of water in a hypothetical, inert, but completely miscible, solvent is gradually increased. Initially only monomers will be present, each molecule having two acid and two base functions available. As dimerization sets in, new species with three acid (H) and three basic (B) sites evolve. Other things being equal, therefore, there will be a higher probability of a monomer interacting with a dimer than with another monomer. Clearly this process, as in all crosslinking polymerizations, would rapidly get out of hand, for the number of reaction sites increases by two on each addition, and soon the growing polymer would scavenge all monomer molecules and other small polymers with great efficiency and become, in effect, a single giant macromolecule.

Other things are not equal, however. First, not all the new sites have the same reactivity as those of the parent molecule, for the reasons outlined. Thus for the dimer, some sites (labelled (1) in Fig. 2) have greatly reduced reactivity (towards H-bonding) whereas others (3) have greatly enhanced reactivity. Second, when cyclic structures (such as Fig. 2*d*) occur,

the number of sites is diminished by two relative to the corresponding open structure. (This fall does not occur on chain branching as in Fig. 2e.)

As the concentration increases, so monomers will add preferentially to sites marked (3). This leads to "normal" linear growth, but once there are four or more molecules in the growing chain, there arises the probability of cyclization. This has a high probability because it comprises a (3)-(3) interaction. (This is another way of saying that the induced $\delta+$ and $\delta-$ charges neutralize in this step.) Once cyclization has occurred, there are no highly reactive sites, the reactivity being comparable with that of the monomer.

Chain branching, like growth from a cyclic structure, has a relatively low probability, but once it has occurred, further growth from the branch site (chain crossing) is encouraged. This reaction site is indicated as (3*) because the induced $\delta+$ or $\delta-$ charges are localized in one function rather than being

tend to form H-bonds at all favourable points of contact. This is bound to involve strongly bent H-bonds in some regions, and unusually long bonds in others.

Some H-bonds will break more readily than others. Of course, those bonds formed between two closely knit units as described in the seventh point above that are forced to be bent or to be very long will break most readily, but even for non-strained structures bond strengths can be defined in terms of the reactivity of the new sites to be formed when the bond breaks. This yields the eighth rule, that the bond will be stronger the greater the net reactivity of the participating groups. Also, ninth, ring structures will have long life times because ring opening leads invariably to (3+) and (3-) centres.

Although I have explained the stability of rings I do not mean to imply that rings such as Fig. 2d will occur with high frequency in water at normal temperatures, for the degree of polymerization is then, I believe, likely to be complete. Within

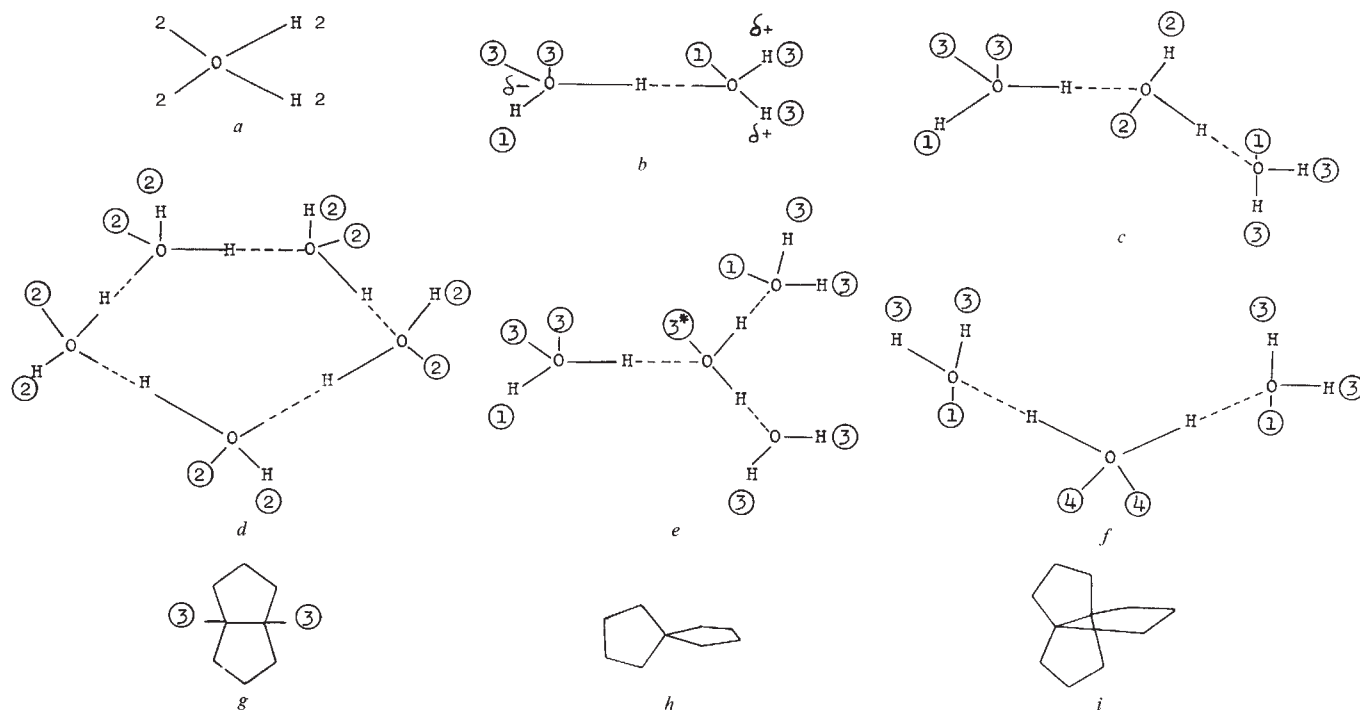


Fig. 2 Various water polymers with relative reactivities. ① < ② < ③ < ④.

distributed over two. If by chance a structure such as Fig. 2f is formed it will generate two exceptionally reactive acidic or basic sites designated (4)—thus if this unlikely type of structure were to appear, it would soon be lost.

Several "rules" arise from these simple considerations. First, interaction between (3)+ and (3)- functions is more probable than that between (3)+ and (2)- or vice versa. In general, (3)+(3) > (3)+(2) > (2)+(2) $\approx$ (3)+(1) > (2)+(1) > (1)+(1), the last two being most improbable. Second, growing polymers generally have several peripheral (3)-sites, so polymer-polymer interactions are very probable. Third, closure to give rings is highly favoured, with 5- and 6-membered rings especially probable, though by no means exclusive. Fourth, chain branching will lead to chain crossing: this, coupled with (4), will encourage multiple ring growth, such as Fig. 2g, h and i. Fifth, once "polymerization" has proceeded far beyond the dimerization stage there will be an overwhelming tendency towards large, three dimensional units, so that pure liquid water at low temperature will, in this sense, be a "single" unit. Sixth, any added monomer could at once be scavenged by one of a myriad of reactive sites. Seventh, mis-matching will abound because when growing units collide they will

the large molecule(s) envisaged, however, such structures will be prevalent as integral parts of the network.

Fluidity will then comprise perpetual regrouping of units. Bonds will bend or stretch, break and reform with neighbours. Tenth, non-bonded lone pairs and protons ($-\text{OH}_f$) are bound to be present, but they represent a limiting case. They should be spectroscopically significant because they represent a precise situation (or turning point) whereas the stretched and bent bonds are smeared over a wide range of possible configurations. Similarly, the fully bonded, unstrained units will be well defined spectroscopically.

Yet another situation that will tend to lower the number of $-\text{OH}_f$ groups can be envisaged. If a non-H-bonded proton is constrained by surrounding structure into a space containing neighbouring H-bonds, but no free lone pairs, then, being electrophilic, it could well move in to form a three centre H-bond as in Fig. 3. Hence I envisage one strong H-bond and one weak one; presumably under balanced circumstances they could be equivalent. This is one possible transition state for H-bond exchange, and it is reminiscent of the structures invoked for electrophilic attack at saturated carbon^{7,8}.

These are the chief conclusions. They appear to render

improbable all those models involving anything resembling ice-like units and monomeric water. They also, in my view, rule out popular concepts involving cooperative rapid break-down or buildup of structural units as in the "flicking cluster" theory. Thus I can discover no set of structures for which breakage of a specific H-bond leads to a significant tendency for others to break sympathetically. Certainly, it will take more energy to open a ring than to break one subsequent bond in a network system, but the gain then stops, and as highly reactive centres (3) are formed, there is a high probability of their reacting again with near neighbours.

Similarly, the probability of bond breaking proceeding to the extent required to form monomeric molecules at normal temperatures is low. Thus, for example, for a four bonded molecule, breaking one bond at once generates a (3*) site which would react strongly with any dangling bond in the vicinity, reforming the four coordinated molecule.

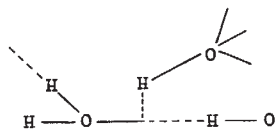


Fig. 3 Possible weak interaction between a non-bonded water proton and a lone pair of electrons already hydrogen bonded.

If a non-polar entity of molecular dimensions were to be inserted into water, a surface of some sort must be formed around it. Water molecules can do this for many such objects, especially if they are near-spherical, while retaining their tendency to be four coordinated. This is demonstrated by the crystalline clathrate which displays a wealth of possible structures in all of which, cavities are beautifully and economically described by hydrogen bonded cages. Growth of a single such cavity, "catalysed" by our spherical object can of course knit in with the rest of the haphazard structures that I have envisaged. Cavities of certain sizes tend to breed more, however, to such an extent that in the crystalline hydrates empty cavities are frequently found. As the concentration of added material rises, one might therefore envisage a tendency for the molecules to cluster together so that, in certain regions, this tendency to propagate cavities is satisfied. This leads to the prediction of both an increase in defined structure for water and a tendency to clustering for the guest molecules (this is, however, a special sort of clustering in which the guest molecules are specifically not in contact, but are separated by a clathrate-like filigree of water molecules). Such effects will be extremely sensitive to temperature and will rapidly diminish on heating.

Investigating Concepts

These concepts have proved most helpful in discussing the properties of aqueous solutions and they can be probed by several techniques, including infrared and Raman spectroscopy, X-ray scattering, and the use of machine models.

In spite of continuing controversy, it now seems clear that $-\text{OH}_f$ groups can be detected by infrared and Raman spectroscopy, most clearly by near infrared "overtone" studies⁹ and somewhat less satisfactorily by Raman studies¹⁰. The reason why infrared studies in the fundamental regions have apparently given negative evidence¹¹, seems to be a question of selection rules. Thus because the concentration of $-\text{OH}_f$ is low, the relative oscillator strength weak in the fundamental region, and the band separation small, the large, broad absorption for H-bonded units completely hides that for $-\text{OH}_f$. The band separation is roughly doubled in the first overtone region,

however, and the H-bonded units lose oscillator strength more than the free groups so that now a narrow $-\text{OH}_f$ band is clearly resolved.

We have just completed a very thorough survey of published infrared data for liquid water under a wide range of conditions (L. M. Kleiss, H. A. Strobel, and M. C. R. S., to be published) in the hope of deciding between the two alternatives that the "free" bands are caused simply by $-\text{OH}_f$ groups or alternatively by free, monomeric, water molecules. We conclude that no such selection can be made with complete confidence, although certain results favour the $-\text{OH}_f$ model. Our new data for H_2O and HOD in the second overtone region ($10,000\text{ cm}^{-1}$) do, however, afford the required proof, and we conclude from these that the concentration of monomeric water in liquid water under normal conditions is undetectably small. This result accords very well with our expectations and with the molecular dynamics calculations of Stillinger and Rahman.

The results show that at low temperatures the number of $-\text{OH}_f$ groups is low, but this increases steadily on warming. It is definitely decreased by various bulky, near-spherical additives such as t-butyl alcohol. This can be taken as indirect evidence in favour of a net increase in the structure of these solutions, as can the unexpectedly large initial high field n.m.r. shifts found at low temperatures for such solutions¹².

Recent X-ray scattering results¹³ show that most water molecules retain their tendency to be tetrahedrally coordinated. The next shell is strongly blurred, however, and there is no evidence for long range order. Furthermore, the liquid is apparently quite homogeneous.

Although statistical calculations are helpful¹⁴, the approach most likely to yield a good picture of the real structure of water is the experimental one in which a computer is used to give information about the behaviour of an ensemble of molecules left to interact at random. The Monte Carlo method has already been applied to the water structure problem¹, but the results so far published are hard to assess. The more powerful "molecular dynamics" procedure has, however, recently been applied by Rahman and Stillinger, and this promises to be most informative. Their preliminary results² do seem to accord rather well with my prognostications. They employ a potential which specifically incorporates a tendency to form tetrahedral arrays of linear H-bonds which is based on complete MO calculations for the dimer. They conclude, in particular, that there is no evidence at any temperature in favour of models in which two principal types of water molecules are postulated, namely bonded water and monomeric water. This accords well with the infrared results described above. There is evidence, however, for the frequent occurrence of $-\text{OH}_f$ groups and, of course, of non-H-bonded "lone pairs" of electrons.

I thank Dr M. J. Blandamer and other collaborators for many helpful discussions.

¹ Barker, J. A., and Watts, R. O., *Chem. Phys. Lett.*, **3**, 144 (1969).

² Rahman, A., and Stillinger, F. H., *J. Chem. Phys.*, **55**, 3336 (1971).

³ Del Bene, J., and Pople, J. A., *J. Chem. Phys.*, **52**, 4858 (1970).

⁴ Hankins, D., Moscovitz, J. W., and Stillinger, F. H., *J. Chem. Phys.*, **53**, 4544 (1970).

⁵ Magnusson, L. B., *J. Phys. Chem.*, **74**, 422 (1970).

⁶ Eisenberg, D., and Kauzmann, W., *The Structure and Properties of Water* (Clarendon Press, Oxford, 1969).

⁷ Olah, G. A., Halpern, Y., Shen, J., and Mo, Y. K., *J. Amer. Chem. Soc.*, **93**, 1251 (1971).

⁸ Lewis, E. S., and Symons, M. C. R., *Quart. Rev.*, **12**, 230 (1958).

⁹ Worley, J. D., and Klotz, I. M., *J. Chem. Phys.*, **45**, 2868 (1966).

¹⁰ Walrafen, G. E., *J. Chem. Phys.*, **48**, 244 (1968).

¹¹ Yellin, W., and Courchene, W. L., *Nature*, **219**, 852 (1968).

¹² Symons, M. C. R., and Blandamer, M. J., in *Hydrogen-Bonded Solvent Systems* (edit. by Covington, A. K., and Jones, P.), 211 (Taylor and Francis, 1968).

¹³ Narten, A. H., and Levy, H. A., *Science*, **165**, 447 (1969).

¹⁴ Levine, S., and Perram, J. W., in *Hydrogen Bonded Solvent Systems* (edit. by Covington, A. K., and Jones, P.), 115 (Taylor and Francis, 1968).

Border and Colour Coding in the Retina of the Frog

TOM REUTER & KAJ VIRTANEN

Department of Physiological Zoology, University of Helsinki, Helsinki 10

The behavioural evidence for colour discrimination in the frog can be explained on the basis of apparent colour interactions between the retinal ganglion cells.

ACCORDING to Granit's¹ dominator-modulator theory some optic nerve fibres transmit brightness information while others are specialized for colour. This fibre specialization seems to be an experimental fact at least in so far as that many optic nerve fibres¹⁻⁴ and lateral geniculate cells⁵⁻⁸ in the mammalian visual system lack colour coding properties. But some electrophysiological^{6,9,10} and psychophysical^{11,12} results indicate the existence of neurones which transmit information about both colour and brightness patterns (optic nerve fibres code for intensity differences, brightness patterns, brightness borders, rather than for absolute light intensity). The fact that non-colour-coding fibres are quite rare in the goldfish retina¹³ points in the same direction.

The eye of the frog provides a good opportunity to study this problem. The retinal ganglion cells and optic nerve fibres of this animal separate into four or five classes, that respond in different and highly specific ways to moving dark or bright objects of varying sizes¹⁴⁻¹⁶. We refer here to this classification as the Maturana *et al.* classes¹⁻⁴. The roles played by these in the frog's analysis of its visual world are quite well understood as regards brightness^{17,18}. If one or several of them could be shown to serve colour discrimination as well, these cells would constitute good examples of neurones transmitting information about both brightness patterns and colour.

Ten years ago Muntz^{19,20} found that the frog's (*Rana pipiens* and *R. temporaria*) main colour discriminating behaviour, that is its specific orientation towards blue, seems to be mediated by a separate class of blue-sensitive on fibres (responding to the on-set but not the off-switch of illumination). These pure on fibres were found to project to the diencephalon, an observation which indicates an extreme specialization of the visual system of the frog with separate colour coding fibres running to the diencephalon, while all classes 1 to 4 are known to pass to the tectum¹⁶.

This cannot, however, be the whole truth. Some years ago Liberman⁹, who studied the responses of single optic nerve fibres of the frog to blue and red stimuli, concluded that a considerable fraction of the on-off fibres (which apparently run to the tectum) transmit both brightness and colour information. And more recently Reuter²¹ has described fibres which receive on responses from the blue-sensitive green rods and off and on-off responses from the yellow-sensitive cones. He found that these two inputs interact in an opponent colour manner typical for colour discriminating neurones in the vertebrate retina.

Our aim was to study the correlation between these opponent colour cells and the classes 1 to 4. We have found that at

least 80% of the ganglion cells of the retina of the frog (*R. temporaria*), in fact representatives of classes 1 to 4, and with the possible exception of the rare class 5, receive inputs from both green rods and cones and show some kind of opponent colour interaction.

The methods used were basically the same as those described by Donner and Reuter²²⁻²⁴ and Reuter²¹. Frogs (*Rana temporaria*) light-adapted overnight at room temperature were decapitated and the eyes excised and opened. Isolated and opened frog eyes seem to function normally during several hours at the temperatures used in these experiments (11-13°C). Spike potentials were recorded extracellularly with glass micro-pipettes from single fibres or ganglion cell bodies close to the retinal surface. The responses were amplified and displayed in the usual manner and occasionally recorded on film. The stimulating light reached the eye through two channels, one for steady background illuminations, the other for signals in the form of bright spots of varying size, intensity and colour (interference filters), or dark spots and boundaries movable against an illuminated background. All stimuli were well focused on the retina.

When a stable recording from an isolated unit was obtained and the receptive field of the cell had been located on the retina, the experiment was continued in two steps. First, the cell type was determined according to the Maturana *et al.* classification with the aid of moving dark and bright spots and bars, and with on-off stimuli with small spots and large fields covering the whole retina. This first step was performed using yellow lights, which stimulate the cones but not the green rods²².

During the second step the spectral sensitivity and possible opponent colour characteristics of the unit were determined by measuring the on and off thresholds (the least intensities producing discharges of one or several impulses) at a number of wavelengths. All threshold were measured using 5 s stimuli with a small spot of light covering the centre of the receptive field. A steady yellow background illumination kept the red rods saturated²⁴, and facilitated the study of the green rod contribution by selectively depressing the cone sensitivity^{21,22}. Further it tended to minimize occasional spontaneous activity.

Fig. 1 shows the spectral sensitivities of four cells representing classes 1-4. The responses of these cells to brightness patterns fitted the description by Maturana *et al.*¹⁶ very well, and can be described as follows: the class 1 and 2 cells responded with an on and off burst to a properly positioned spot of light, but did not respond when a large field covering the whole retina was turned on and off. The class 1, but not the class 2 cell, responded well to moving straight edges and bright spots (except when moved in the nasal direction on the retina), but both of them responded vigorously to a moving dark spot. If the dark spot stopped in their receptive fields both cells produced maintained discharges, which stopped when all the light was transiently turned off for a fraction of a second. After this the response of the class 1 cell reappeared, but the class 2 cell remained silent.

The class 3 cell responded with short high frequency on-off bursts to large and small fields (especially short to large fields), and responded to the slightest displacement within the receptive

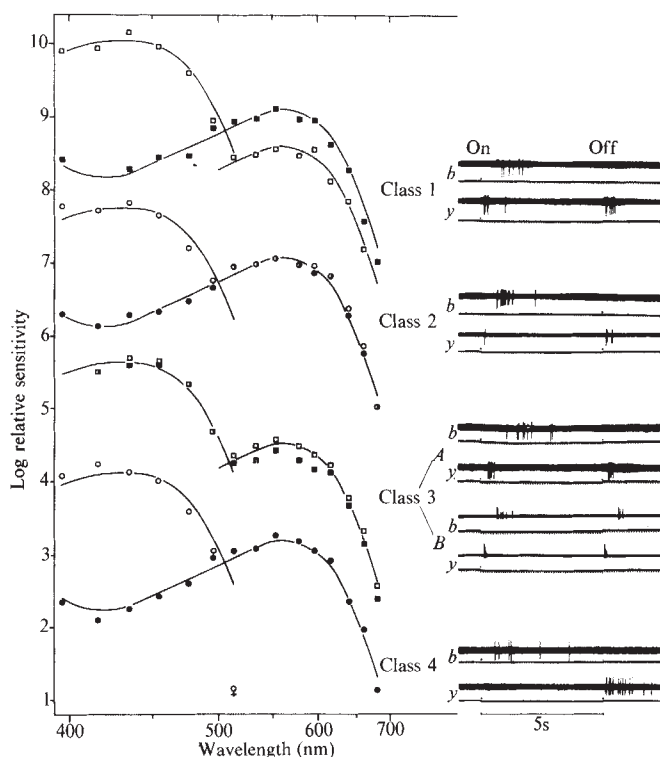


Fig. 1 Left. Spectral sensitivity (reciprocal of the quantum intensity at threshold) of four cells representing classes 1–4. Open symbols: on thresholds. Solid symbols: off thresholds. Stimuli: 5 s exposures to light spots (0.3 mm diameter on the retina) of varying wavelengths from 396 to 681 nm. Background: 564 nm, 3×10^{11} quanta $\times$ mm $^{-2}$ s $^{-1}$ reaching the retinal surface. The curves peaking at 433 and 560 nm represent the spectral sensitivities of the green rods and the cones respectively (explanation in text). The four experiments are displaced vertically for clearness. The open circle with a downward arrow belonging to the class 4 cell indicates that the on threshold at 513 nm is lacking (suppressed by cones) or lies below this level. Right. Filmed recordings showing the responses of five cells representing classes 1–4 (2 representatives of class 3, one (A) without and the other (B) with a green rod-mediated off response). Each cell was exposed both to a blue (b, 476 nm) and a yellow-green (y, 553 nm) spot 0.6 log units above the thresholds. 5 s stimuli, on and off marked by small dots between the time records and the actual impulse recordings. 564 nm background as above.

field of an edge or small spot. Differing from classes 1 and 2 it gave stronger reactions to rapid movements and never continued to respond when a dark spot stopped in the receptive field. The class 4 cell responded with prolonged discharges to every decrease in the illumination of the receptive field, especially long when a large field was turned off. It never responded to an increase in the intensity of the yellow light used for these tests, and never with maintained responses to standing edges.

In Fig. 1 we have correlated two types of curves with the spectral sensitivities of the on (open symbols) and off (solid symbols) thresholds of these four cells. The curves peaking at 433 nm represent the absorbance spectrum of the visual pigment of the green rods^{22,25,26}, while the curves with a peak at 560 nm and a small secondary rise at 400 nm represent the combined cone sensitivity, that is the “photopic dominator” of the frog^{21,27}. The retina of the frog contains single cones with a pigment absorbing maximally at 575 nm and double cones with one 575 nm component and one accessory component with a 502 nm pigment²⁶, but under the experimental conditions used here these cone types seem to cooperate forming a remarkably stable spectral sensitivity curve of the type shown in Fig. 1^{21,28}.

It is obvious that the class 1 and 2 cells in Fig. 1 receive only on inputs from the green rods, but on-off inputs from the cones. In the class 3 cell both receptor types contribute both

to on and off. A characteristic observation when studying the green rod contributions to the class 3 cells was, however, that the on response often dominates the off, which could sometimes be elicited only in the threshold region, and sometimes not even there. This is shown by the filmed recordings on the right in Fig. 1 showing the responses of five cells, representing classes 1–4, to 5 s exposures to blue (b) and yellow-green (y) spots. The intensities of these spots were 0.6 log units above the thresholds and the blue spots stimulated only the green rods, the yellow-green only the cones. Two representatives of class 3 are shown; in one (A) the off response of the green rods is absent, in the other (B) it is present.

Table 1 Discharge Characteristics of Green Rod- and Cone-mediated Responses

	Discharge length (s)	Discharge latency (s)	Discharge frequency impulses s $^{-1}$
Green rod-mediated responses	0.96 ± 0.26	0.70 ± 0.04	11.2 ± 2.1
Cone-mediated responses	0.16 ± 0.05	0.17 ± 0.01	65.8 ± 7.4

Discharge lengths, latencies and frequencies (means and standard errors) for thirteen green rod-mediated and twenty-four cone-mediated on responses. Stimuli: 5 s exposures to blue (476 nm) and yellow-green (553 nm) spots 0.6 log units above the thresholds. Yellow background illumination. In all three respects the responses mediated by the green rods differ significantly from the cone-mediated responses ($P < 0.01$ to $P < 0.001$), although the average number of impulses per discharge was nearly the same for both types of responses.

It is evident that stimulation of the green rods results in longer discharges with longer latencies and lower frequencies than stimulation of the cones (see also Table 1). These differences between the responses of the frog's retina to blue and yellow lights have been observed by several authors in a wide variety of background and stimulus conditions^{9,19,21,29,30}.

The opponent interaction between the cones and the green rods does not appear only in the difference between the on responses from the green rods and the off or on-off responses from the cones. The cone signals also tend to suppress the prolonged green rod responses²¹. This effect is shown in Fig. 2, where both a class 1 and a class 2 cell have been exposed to blue (476 nm) stimuli of two intensities. The lower intensity stimulates only the green rods, the higher reaches the cone threshold with the result that the long low frequency responses from the green rods are replaced by typical short cone discharges. In the class 1 unit the cone-mediated on threshold is not reached, but the on response from the green rods is completely suppressed. This kind of opponent interaction is observed also in class 3 cells, and in class 4 cells it typically results in a complete suppression of the on response. The dominance of the cone system over the green rods is also shown by the fact that white lights elicit only the cone-mediated response pattern¹⁶.

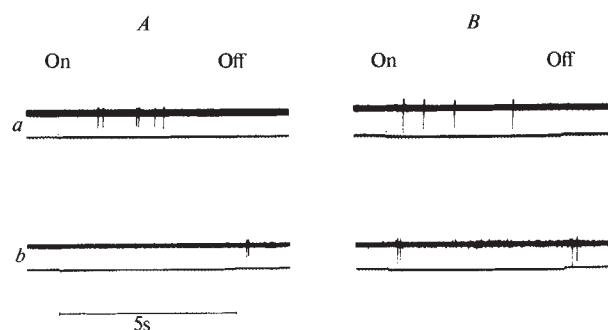


Fig. 2 Responses of two cells representing classes 1 (A) and 2 (B) to 5 s stimuli with 476 nm spots of two intensities: a, the lower intensity (0.8 log units above the on threshold); b, the higher (1.4 log units above the on threshold). Yellow background illumination.

Table 2 Summary of Response Types

Class of cell	Green rod on cone on	Green rod on cone on-off	Green rod on cone off	Green rod on-off cone on-off	Green rod on-off cone off	Cone on-off	Cone off	Totals
1		15	3			1	1	20
2		16	1			5	2	24
1 or 2		10	2			7	2	21
3		9				3		39
4			17		27		3	21
5					1		2	2
Pure on	2							2
Totals	2	50	23	27	1	16	10	129

Summary of the response types of all 129 cells, which have been characterized both with regard to their Maturana *et al.* class and their types of inputs (on, on-off, off) from the green rods and the cones. The figures give the number of cells belonging to each category. Twenty-one cells are named "class 1 or 2", because these two classes are not always separable¹⁶. Figures in heavy print mark the types of responses most frequently observed. The main deviations from the typical response patterns are due to the lack of green rod inputs to 20% of the cells, and to the fact that some class 3 cells did not respond with green rod-mediated off responses and some class 1 and 2 cells not with cone-mediated on responses to the stimuli used.

Fig. 1 illustrates the types of response most frequently observed. A summary of the response types of all 129 cells, which during this study have been examined in detail, is given in Table 2. Two cells were of the pure on type, and in agreement with the description by Muntz¹⁹ both the green rods and cones were found to contribute to the reactions of these cells. Two units showed the characteristics of the rare class 5, which is supposed to code for general light intensity¹⁶. Both of them were without green rod inputs and thus devoid of opponent colour characteristics, which may be interpreted to indicate that this class code only for brightness and not for colour.

In twenty-six cases (20% of the units) no green rod responses were observed. This was at least partly due to the fact that the green rod system is remarkably delicate. The disappearance of the contribution from green rods during the course of an experiment is often the first sign of deterioration of a preparation. It is significant that the characterization of the units without green rod inputs were started on an average 133 ± 14 (standard error) min after the dissection of the eye, compared with 85 ± 6 min for the rest of the units (the difference is significant, $P < 0.01$). It remains an open question whether or not some units are normally without inputs from green rods.

Clearly, using violet and red stimuli it is possible to map separately the receptive fields of the green rod and cone contributions (Fig. 1). This has been done using small spots of light at different positions on the retina, and by determining the area-threshold relation (for methods see Hartline³¹, Barlow¹⁵, Reuter²¹). It was found that the ganglion cells receive green rod inputs from a larger retinal region than they receive cone inputs, with the result that the periphery of their excitatory receptive fields is dominated by green rods and thus is blue-sensitive, while, in normal conditions without a strong yellow background, the centre is dominated by cones and thus is yellow-sensitive (A.-C. Bäckström and T. R., in preparation). These experiments showed also that both green rods and cones contribute to the lateral inhibition surrounding the excitatory receptive fields of class 1, 2 and 3 cells.

Thus the chromatic properties of the receptive fields of the frog's ganglion cells resemble very much the opponent colour centre-surround organization observed in the retinas of several other vertebrates^{4,10,13,32,33}.

We may ask to what extent the characteristics of the retinal ganglion cells of the frog correlate with the colour discrimination capacity of this animal. *R. temporaria* has been shown to possess a true colour discrimination in at least two experimental situations: (1) Birukow³⁴, studying the optomotor response to the rotation of a cylinder with blue and grey stripes, found that no shade (intensity) of grey matched the blue so well that the frogs did not react to the rotation. A match was possible, however, using grey and green stripes,

which agrees with the fact that the frog seems to be dichromatic and may have a neutral point somewhere in the green (see Fig. 1). (2) Muntz²⁰, studying the positive phototaxis to the blue, found that the frogs in a choice situation prefer "pure" blue to a mixture of the same blue with a green light. The addition of green light made the blue less attractive.

In Burikow's experiment it is not clear whether the frogs "know" what stripe is blue and what is grey. It is sufficient to suppose that the retinal ganglion cells respond when a "colour border" (a boundary between two fields of different colours but the same brightness) passes over their receptive fields, and in fact it is obvious that many ganglion cells will respond, because their on and off components have different spectral sensitivities and an intensity of grey which keeps the on component equally stimulated and silent during the colour shifts will change the stimulation of the off component and vice versa²¹. It has also been shown in direct experiments that many ganglion cells in the frog's retina respond to colour changes within a stationary spot of light independent of the relative intensities of the two colours³⁵⁻³⁷.

Muntz's phototaxis experiments show that the frogs not only detect colour borders, they also know what side of the border is blue and what grey or green. One may speculate about what of the characteristics of the green rod responses are crucial for the frog's identification of the blue part of the spectrum. It is tempting to think that the prolonged low frequency responses to an increased intensity in the blue are important, because they are common to all classes 1-4. Further, the observation that an addition of green light makes the blue less attractive for the frog correlates well with the fact that an addition of cone activity cuts off the long green rod responses (Fig. 2). The idea that information about colour may be given by the distribution of impulses in time has been proposed by Donner³⁸, who studied the cat retina, and by Liberman⁹, who specifically referred to the prolonged responses of the retinal ganglion cells of the frog to blue stimuli.

Our experiments suggest the existence in the frog's retina of separate channels for green rod responses. When during dark adaptation the red rods begin to contribute they enter the on-off and off channels of the class 1, 2 and 4 cells, that is the cone but not the green rod channels³¹. This makes possible colour vision in the mesopic state. The red rods simply contribute to the cone side of the opponent colour system. Further, this arrangement, together with the fact that the cone channels dominate and are sufficient for eliciting all the Maturana *et al.* characteristics, guarantees the invariance of coding of brightness pattern information during the shift from photopic to scotopic vision.

The main role of the green rod channels seems to be to "add colour to the system". We do not know how the brain of the frog integrates and uses the information we observe

in the discharges of single fibres, but our results indicate that most optic nerve fibres of *R. temporaria*, and not only the rare pure on fibres described by Muntz¹⁹, transmit enough information to explain the dichromatic colour discrimination capacity of this animal.

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- ¹ Granit, R., *Sensory Mechanisms of the Retina* (Oxford University Press, London, 1947).
- ² Hubel, D. H., and Wiesel, T. N., *J. Physiol.*, **154**, 572 (1960).
- ³ Michael, C. R., *J. Neurophysiol.*, **31**, 249 (1968).
- ⁴ Gouras, P., *J. Physiol.*, **199**, 533 (1968).
- ⁵ De Valois, R. L., Abramov, I., and Jacobs, G. H., *J. Opt. Soc. Amer.*, **56**, 966 (1966).
- ⁶ Wiesel, T. N., and Hubel, D. H., *J. Neurophysiol.*, **29**, 1115 (1966).
- ⁷ Pearlman, A. L., and Daw, N. W., *Science*, **167**, 84 (1970).
- ⁸ De Valois, R. L., and Pease, P. L., *Science*, **171**, 694 (1971).
- ⁹ Liberman, E. A., *Biophysics*, **2**, 424 (1957).
- ¹⁰ Spekreijse, H., Wagner, H. G., and Wolbarsht, M. L., *J. Neurophysiol.*, **35**, 73 (1972).
- ¹¹ McCulloch, C., *Science*, **149**, 1115 (1965).
- ¹² Held, R., and Shattuck, S. R., *Science*, **174**, 314 (1971).
- ¹³ Daw, N. W., *J. Physiol.*, **197**, 567 (1968).

- ¹⁴ Hartline, H. K., *Amer. J. Physiol.*, **121**, 400 (1938).
- ¹⁵ Barlow, H. B., *J. Physiol.*, **119**, 69 (1953).
- ¹⁶ Maturana, H. R., Lettvin, J. Y., McCulloch, W. S., and Pitts, W. H., *J. Gen. Physiol.*, **43**, 129 (1960).
- ¹⁷ Grüsser, O.-J., and Grüsser-Cornehls, U., *Z. Vergl. Physiol.*, **59**, 1 (1968).
- ¹⁸ Grüsser, O.-J., and Grüsser-Cornehls, U., in *Verhandlungen der Deutschen Zoologischen Gesellschaft*, **64** (edit. by Rathmayer, W.) (Gustav Fischer Verlag, Stuttgart, 1970).
- ¹⁹ Muntz, W. R. A., *J. Neurophysiol.*, **25**, 699 (1962).
- ²⁰ Muntz, W. R. A., *J. Neurophysiol.*, **25**, 712 (1962).
- ²¹ Reuter, T., *Acta Zool. Fennica*, **122**, 1 (1969).
- ²² Donner, K. O., and Reuter, T., *Vision Res.*, **2**, 357 (1962).
- ²³ Donner, K. O., and Reuter, T., *Vision Res.*, **5**, 615 (1965).
- ²⁴ Donner, K. O., and Reuter, T., *J. Physiol.*, **199**, 59 (1968).
- ²⁵ Dartnall, H. J. A., *Vision Res.*, **7**, 1 (1967).
- ²⁶ Liebman, P. A., and Entine, G., *Vision Res.*, **8**, 761 (1968).
- ²⁷ Granit, R., *Acta Physiol. Scand.*, **3**, 137 (1942).
- ²⁸ Donner, K. O., *J. Physiol.*, **149**, 318 (1959).
- ²⁹ Orlov, O. Yu., *Biophysics*, **6**, 48 (1961).
- ³⁰ Frank, R. N., *Vision Res.*, **10**, 1101 (1970).
- ³¹ Hartline, H. K., *Amer. J. Physiol.*, **130**, 700 (1940).
- ³² Wagner, H. G., MacNichol, jun., E. F., and Wolbarsht, M. L., *J. Gen. Physiol.*, **43**, 45 (1960).
- ³³ Michael, C. R., *J. Neurophysiol.*, **31**, 268 (1968).
- ³⁴ Birukow, G., *Z. Vergl. Physiol.*, **27**, 41 (1939).
- ³⁵ Bongard, M. M., and Smirnov, M. S., *Biophysics*, **2**, 328 (1957).
- ³⁶ Donner, K. O., and Rushton, W. A. H., *J. Physiol.*, **149**, 288 (1959).
- ³⁷ Scheibner, H., and Baumann, Ch., *Vision Res.*, **10**, 829 (1970).
- ³⁸ Donner, K. O., *Acta Physiol. Scand.*, **21**, Suppl. 72 (1950).

The Origin of the Moon

DON L. ANDERSON

Seismological Laboratory, California Institute of Technology, Pasadena, California 91109

Explanation of the Moon's composition in a model for the condensation of the planets from a cloud of solar composition suggests that the Moon accreted at higher temperatures and lower pressures than the terrestrial planets. This may be explained if the lunar orbit initially had a high inclination.

RECENT studies of the dynamics and thermodynamics¹⁻⁵ of the early solar nebula have provided a basis for a theory of the origin of the Moon which is consistent with presently developed views of the origin of the Solar System. The immediate objective of this article is to extend the hypothesis of inhomogeneous planetary accretion⁵. I suggest that the anomalous properties of the Moon, such as its enrichment in Ca, Al, Ti and other refractories and its depletion in iron and volatiles, can be explained if the bulk of the Moon represents a high temperature condensate.

Condensation of the Solar System

I start from the commonly held view that the solid objects in the Solar System formed by condensation in a cooling disk-shaped nebula. According to Hoyle and Wickramasinghe¹ the surface temperature of the Sun during the over-luminous convective stage is maintained between 3,000 K and 3,500 K during most of the contraction process but eventually reaches ~4,000 K as the main sequence is approached. The planets probably represent a small fraction of the original planetary material which is supposed to be of solar composition.

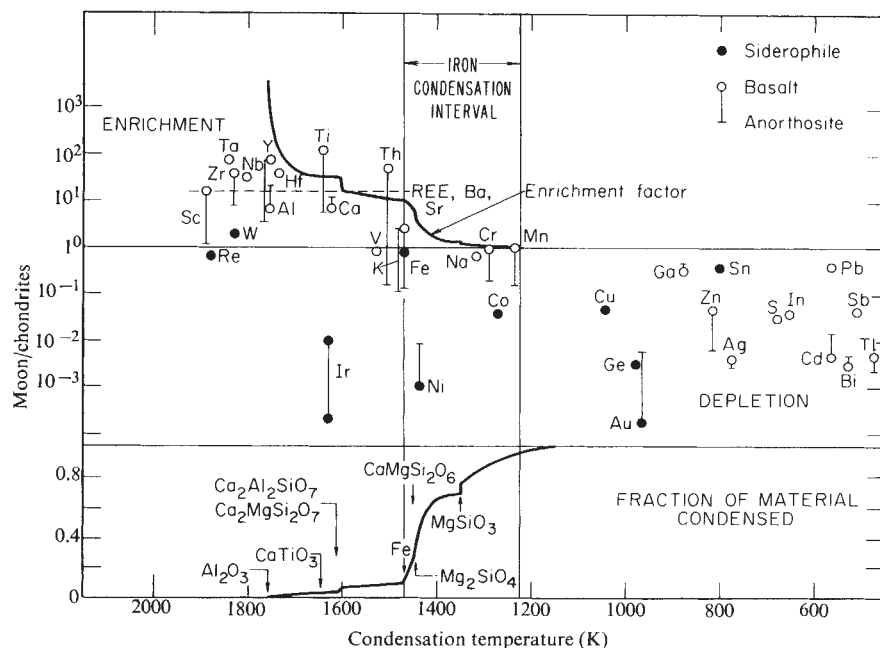
Within the disk the temperature falls off roughly as the square root of distance and different substances condense at distances and times which depend on their concentrations and vapour pressures. Thus the refractory silicates and iron condense in the inner part and the volatiles in the outer part of the Solar System. Hoyle and Wickramasinghe suppose that the accretion of the Earth was substantially complete during the ~10⁴ yr of the high temperature phase and that the volatiles were a later addition, being brought in by debris during the period of ~10⁷ yr required for the Sun to reach the main sequence.

Opacity, density, pressure and temperature in the nebula vary with distance from the Sun and also with height above the median plane. This implies that at any stage during the condensation process, the concentration and composition of the condensed material will be a function of location. Once condensation has started, accretion into planets may keep pace with subsequent cooling and condensation^{1,5}, and, indeed, the composition of the bodies in the Solar System is consistent with their having been formed from material that condensed to a given point in the condensation sequence. Planets accreting in high pressure, or low temperature, regions of the nebula will be further advanced at any given time than those accreting in low pressure, high temperature regions.

Composition of Moon and Earth

How are the differences of composition between the Moon and the Earth to be explained on the nebular condensation theory? I now suggest that the special features of the Moon arise because of the strong dependence of condensation temperature on pressure in the nebula, and the rapid decrease in pressure away from the median plane of the disk. The inclination of the orbits of the Moon and pre-lunar material must have been greater in the past and decreased with time owing to gas

Fig. 1 Chondritic normalized lunar abundances¹⁴⁻¹⁷ as a function of condensation temperature²⁻⁴ of the element or the first condensing compound containing this element at 10^{-3} atmospheres total pressure. Ratios greater than unity indicate enrichment and less than unity indicate depletion.



drag and tidal interactions. It is even possible that at the time of formation the plane of the Moon's orbit was nearly perpendicular to the ecliptic^{6,7}, and within the framework of the nebular condensation theory, the composition of the Moon was determined by the processes of condensation occurring at a distance from the Sun similar to that of the Earth's orbit but at the low pressures obtaining at some distance from the plane of the ecliptic.

The results of analysis on lunar samples indicate that the Moon is enriched in such refractories as Ca, Al, Ti and the rare earth elements and depleted in elements more volatile than iron. Fig. 1 gives chondritic-normalized lunar abundances as a function of condensation temperature. The condensation temperatures^{2,3} are for the element or the first condensing compound containing this element. The condensation temperatures of the REE, Ba and Sr are unknown but the average enrichment of these elements, shown by the dashed line, is about the same as other refractories. In the lower part is shown the fraction of available material that has condensed as a function of temperature³. The initiation of condensation of various compounds is indicated by arrows. Note the rapid increase in the fraction of material which has condensed as the temperature drops to the condensation temperature of iron and the magnesium silicates.

The enrichment factor in Fig. 1 is the reciprocal of the condensed fraction. It indicates the expected enrichment of the trace refractories in the condensed phase relative to chondritic abundances. The refractories are enriched in the Moon to about the level expected in the pre-iron condensates. As the temperature decreases below the condensation temperature of iron the enrichment factor rapidly approaches unity, that is chondritic abundances.

Content of Moon

I propose that the Moon is composed chiefly of compounds that condense before iron and that the volatile content of the Moon was brought in as a thin veneer after the solar nebula dissipated. The latter part of this proposal is not new; Ganapathy *et al.*⁸ have suggested that the volatiles were brought to the surface by chondritic material. A refractory outer layer was proposed^{9,10} to explain some aspects of the geochemistry of the lunar surface material but the present proposal is novel in that it suggests that the whole Moon is enriched in refractories such as Ca, Al, Ti, REE, Ba, Sr and U. The bulk composition may be similar to the Ca-Al rich inclusions in Type III carbonaceous chondrites¹¹ which are thought to represent the early

condensate of the cooling nebula. There is trace element support for this conjecture. Fig. 2 shows the chondritic normalized abundances of Ba, Sr and the REE in a lunar basalt, a lunar anorthosite and the Ca-Al rich inclusions of the Allende meteorite. If lunar basalts and anorthosites were ultimately derived from extensive melting of a source region having the composition of the Allende inclusions, a mixture of the two should yield a level pattern and approximately the same abundances as the inclusions. These constraints are met with the mixture shown. The average enrichment of both the mixture and the Allende inclusions is 16x chondritic, about that predicted on the basis of Fig. 1. A Ca-Al rich interior has been dismissed on the grounds that the high pressure assemblage of such material would be greater than the mean density of the Moon. If, however, a large part of the Moon is above the equilibrium temperature of the high pressure phases, this argument no longer applies.

The accretion during condensation hypothesis seems capable of explaining the differences between the Earth and the Moon

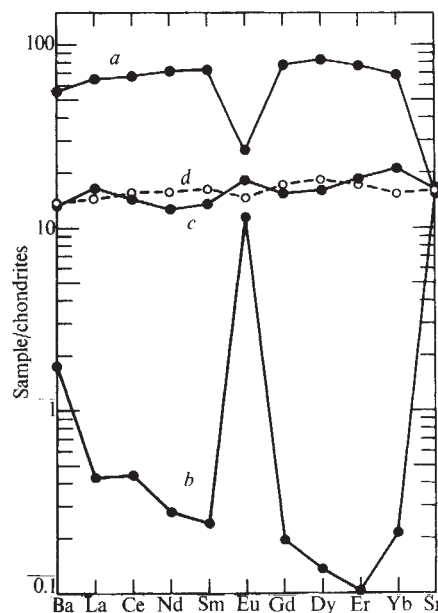


Fig. 2 Chondritic normalized refractory trace elements for lunar basalt (a)¹⁴, lunar anorthosite (b)^{16,17}, Ca-Al rich inclusions from the Allende meteorite (c)¹³, and a mixture of 0.22 basalt + 0.78 anorthosite (d).

even if they formed at the same distance from the Sun. Condensation temperatures depend on pressure as shown in Fig. 3. The pressure in the nebula varies with distance from the Sun and with height above the median plane. The condensation intervals of the refractories, forsterite, and iron overlap at high pressures and diverge at low pressures. The line labelled 1 AU is the temperature in the vicinity of the Earth's orbit during the high luminosity phase of the Sun¹. If the Earth accreted in a dense, high pressure part of the nebula, such as the median plane, it would have iron and the magnesium silicates available for incorporation into its interior as well as the early condensing refractories. Away from the median plane the temperature remains almost constant (A. G. W. Cameron and M. R. Pine, unpublished) but the gas pressure decreases. Condensation, therefore, occurs at lower temperatures, and, in a cooling gas, at later times. As represented in Fig. 3, the Earth will be more than 50% assembled before the Moon starts and will have swept up most of the iron. After the Moon nucleates the Earth will still get most of the remaining iron and later condensates, since it spends all of its time in the median plane. Material condensing off the median plane will initially be in inclined orbits which get tilted toward the plane by gas drag and collisions.

If accretion was rapid and efficient the Moon may have been, and may still be, a chemically zoned body. In order to provide a Ca-Al rich source region with high abundances of refractory trace elements, it has been proposed⁹ that at least the outer shell is enriched in the refractories, including Ca, Al, Sr, Ba, U and the REE. In the present model the interior is devoid of iron and the iron content increases with radius. The low-pressure, high temperature mineralogy of the deep interior would initially be perovskite, diopside and spinel grading into anorthite and ferromagnesian silicates at shallower depths. The drop in electrical conductivity of the Moon at 250–350 km depth¹² is most reasonably interpreted as a sudden decrease in iron content at this depth, a possible relic of the initial layering or the result of iron enrichment in a residual melt. The mean density and the moment of inertia can be satisfied with the Ca-Al rich compositions discussed here. The low electrical conductivity of iron-free silicates leads to higher interior temperatures than inferred by Sonett *et al.*¹².

Overall Model

The lunar igneous rocks and, by implication, their source regions¹³, are enriched in refractories and depleted in elements more volatile than iron. The enrichment of refractories is consistent with that expected for the pre-iron condensates in a partially condensed cloud of solar composition. The volatile content can be explained by a small increment of carbonaceous material brought in after the main accretion⁸. I propose that the bulk of the Moon is composed of material that condensed prior to the condensation of iron. This can be accomplished if the Moon accreted from material that condensed at much higher temperatures or at much lower pressures than the other terrestrial planets and the chondritic meteorites. One likely source for this material is outside the plane of the ecliptic and this favours a lunar orbit of initially high inclination.

Because it accreted in a relatively high pressure part of the nebula, the median plane, the Earth starts to accrete earlier and grows faster than bodies which accrete in highly inclined orbits. Most of these latter bodies are brought into the median plane and swept up by the Earth. The composition of the Moon can be understood if it formed relatively late, after most of the condensable material near the median plane had been swept up by the Earth. This delay in the start of formation of the Moon may be a consequence of the strong pressure dependence of the condensation temperature. The condensation temperatures of the early condensing refractories, iron and the magnesium silicates increase and converge as the pressure increases. This alone can qualitatively account for the differences in size and composition of the Earth, Mercury, Venus and the

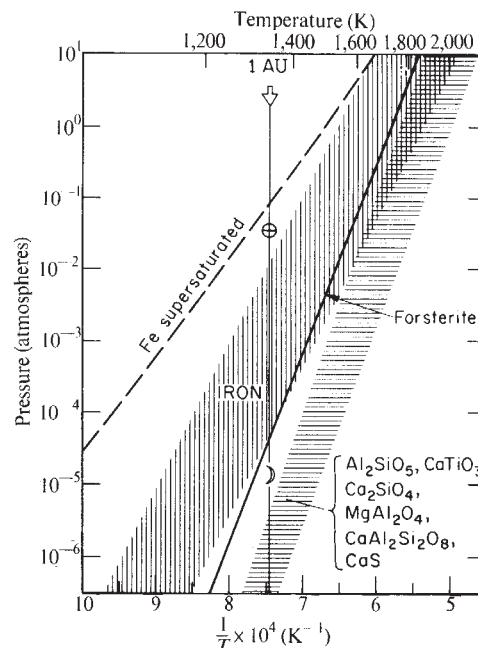


Fig. 3 Condensation temperature versus pressure in the solar nebula^{2,3,18,19}. The temperature at 1 AU is appropriate for a solar surface temperature of 3,000 K (ref. 1). The Earth and the Moon are plotted at the pressure which will explain their bulk compositions. I assume that the Earth and the Moon accreted from material that condensed while cooling from high temperatures to the temperature shown.

Moon. Another effect sets in when a planet grows to sufficient size to retain an atmosphere. Such a planet is more efficient in retaining material that it encounters, and it also triggers condensation in its vicinity. I propose that the high luminosity phase of the Sun prevented complete condensation throughout the disk at 1 AU.

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- 1 Hoyle, F., and Wickramasinghe, N. C., *Nature*, **217**, 415 (1968).
- 2 Larimer, J. W., *Geochim. Cosmochim. Acta*, **31**, 1215 (1967).
- 3 Gossman, L., *Geochim. Cosmochim. Acta* (in the press).
- 4 Clark, jun., S. P., Turekian, K. K., and Grossman, L., in *The Nature of the Solid Earth* (edit. by Robertson, E. C.), 3 (McGraw-Hill, 1972).
- 5 Turekian, K. K., and Clark, jun., S. D., *Earth Planet Sci. Letters*, **6**, 346 (1969).
- 6 Gerstenhorn, M., *Z. Astrophys.*, **26**, 245 (1955).
- 7 Singer, S. F., *Geophys. J. Roy. Astron. Soc.*, **15**, 205 (1968).
- 8 Ganapathy, R., Keays, R. R., Lanl, J. C., and Anders, E., *Geochim. Cosmochim. Acta*, Suppl. 1, 1117 (1970).
- 9 Gast, P. W., and McConnell, jun., R. R., *Third Lunar Science Conference Abstracts*, 257 (1972).
- 10 Wasserburg, G. J., Turner, G., Tera, F., Podosek, F. A., Papanastassiou, D. A., and Hineke, J. C., *Third Lunar Science Conference Abstracts*, 695 (1972).
- 11 Clarke, jun., R. S., Jarosewich, E., Mason, B., Nelen, J., Gomez, M., and Hyde, J. R., *Smithsonian Contrib. Earth Sci. No. 5* (1970).
- 12 Sonett, C. P., Schubert, G., Smith, B. F., Schwartz, K., and Colburn, D. S., *Proc. Second Lunar Science Cong.*, **2**, 2415 (MIT, 1971).
- 13 Gast, P. W., Hubbard, N. J., and Wiesmann, H., *Geochim. Cosmochim. Acta*, Suppl. 1, 1143 (1970).
- 14 Mason, B., and Melson, W. G., *The Lunar Rocks*, 179 (Wiley-Interscience, 1970).
- 15 Paul, J. C., Morgan, J. W., Ganapathy, R., and Anders, E., *Proc. Second Lunar Science Conf.*, **2**, 1159 (MIT, 1971).
- 16 Wakita, H., and Schmidt, R. W., *Science*, **170**, 969 (1970).
- 17 Hubbard, N. J., Gast, P. W., Meyer, C., Nyquist, L., Shih, C., and Wiesmann, H., *Earth Planet. Sci. Letters*, **13**, 71 (1971).
- 18 Lord, H. C., III, *Icarus*, **4**, 279 (1965).
- 19 Blander, M., and Katz, J. L., *Geochim. Cosmochim. Acta*, **35**, 61 (1971).

LETTERS TO NATURE

PHYSICAL SCIENCES

Search for a Trans-Plutonian Planet

Brady and Carpenter¹, when working out an ephemeris for the 1986 apparition of Halley's Comet, found they had to include a secular term to explain the observations of previous apparitions. Kiang² has examined the original records of the apparitions of the comet, and his revised dates of perihelion passage considerably decrease the residuals of Brady and Carpenter's adopted orbit, which includes the secular term. From this term, Brady³ inferred the existence and predicted the position of a trans-Plutonian planet.

During the months of June and July 1972, we conducted a search for the planet, covering an area which extended at least $3\frac{1}{2}^\circ$ from the predicted position in every direction. Two contiguous polygons were covered, bounded by the lines of α, δ given in Table 1.

Table 1 Search Area for Trans-Plutonian Planet

	RA (1950)	DEC (1950)
	h m to h m	° ' to ° '
1	00 15 to 01 40	+67 00 to +74 00
2	00 45 to 01 25	+74 00 to +75 30

Two series of plates of this area were taken on the 13-inch Astrographic Refractor of the Royal Greenwich Observatory, on 'Kodak IIaO' emulsion. The field covered by each plate was about $2\frac{1}{2}$ degrees square, and we allowed $\frac{1}{2}^\circ$ overlap between the plates because of the non-circular images formed away from the centre of the plate. The predicted motion of the planet across the plates was 0.7 mm in 24 h. One day was the minimum interval between plate pairs.

We blinked each plate pair on a blink comparator to the plate limit of the inferior plate of a pair. This in all cases was fainter than magnitude 15.5 and most went fainter than magnitude 16.0. We found no moving object brighter than the magnitude limit on any of the plate pairs.

If Brady's hypothetical planet had the albedo of Pluto and a reasonable density, it would be no fainter than 14 mag. If a trans-Plutonian planet does exist, then either it is a much less massive object and hence considerably fainter than Brady has predicted (see ref. 4), or it is not near Brady's final position.

A. P. O. FOSS
J. S. SHAWE-TAYLOR
D. P. D. WHITWORTH

Royal Greenwich Observatory,
Herstmonceux Castle,
Hailsham,
Sussex

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- Brady, J. L., and Carpenter, E., *Astron.*, **76**, 728 (1971).
- Kiang, T., *Mem. Roy. Astron. Soc.*, **76**, 27 (1972).
- Brady, J. L., *Proc. Astron. Soc. Pac.*, **84**, 314 (1972).
- Seidemann, P. K., *Astron. J.*, **76**, 740 (1971).

Earth Tides and the Triggering of Eruptions from Mt Stromboli, Italy

EARTH tides with stress gradients exceeding 10^{-8} bar/m (ref. 1) and with predominant periods at approximately 12 h, 24 h and 14.7 day constitute the largest short period oscillatory stress in the Earth's crust². The release of secular tectonic strain accumulation in the crust by tidal triggering has been suggested³⁻⁵, and many attempts^{2,3,5-8} have been made to correlate earthquake times with records of Earth tides. All these attempts have proved unable conclusively to relate tides to the occurrence of earthquakes, although microearthquakes may prove to be an exception⁹.

For volcanic eruptions the question of whether the tides provide a triggering mechanism is still unclear, although observations of pre-eruptive strain accumulation^{10,11} indicate a long-term process. Because eruptions of many of the world's volcanoes are usually described in relation to the characteristics of Mt Stromboli, Italy, that volcano was selected as a test case for these ideas. Eruptions during the past 72 yr have been relatively frequent and eruption times and descriptive details of the eruptions¹²⁻¹⁴ were consistently better than the data from other volcanoes. Of the 33 major reported eruptions, all were known to the day and 67% to the hour. 82% of the events could be definitely classified as initial or primary eruptions following quiescent periods of varying intervals from a few months to several years. The remainder of the volcanic activity was either of secondary or tertiary nature. Geochemical analysis of the erupted products¹² indicate all to be of olivine trachybasalt character.

We have derived components of the solid Earth tide as a function of time and position using a program based on a method similar to that developed by Longman¹⁵. These agree with the values from other independent computations¹⁶, and a check with published tidal observations from quartz

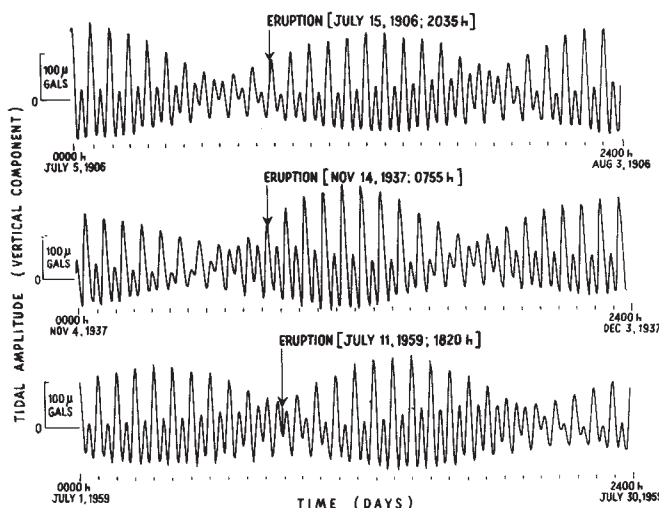


Fig. 1 Three plots of the calculated vertical tidal acceleration as a function of time before and after primary eruptions from the volcano Stromboli. Each starts 10 days before the eruption.

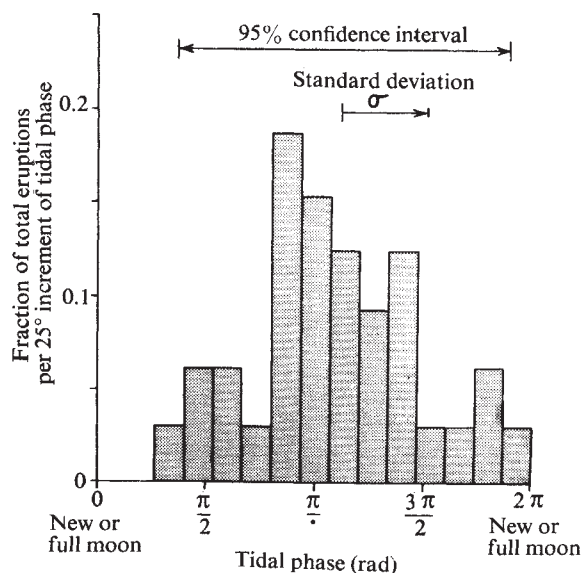


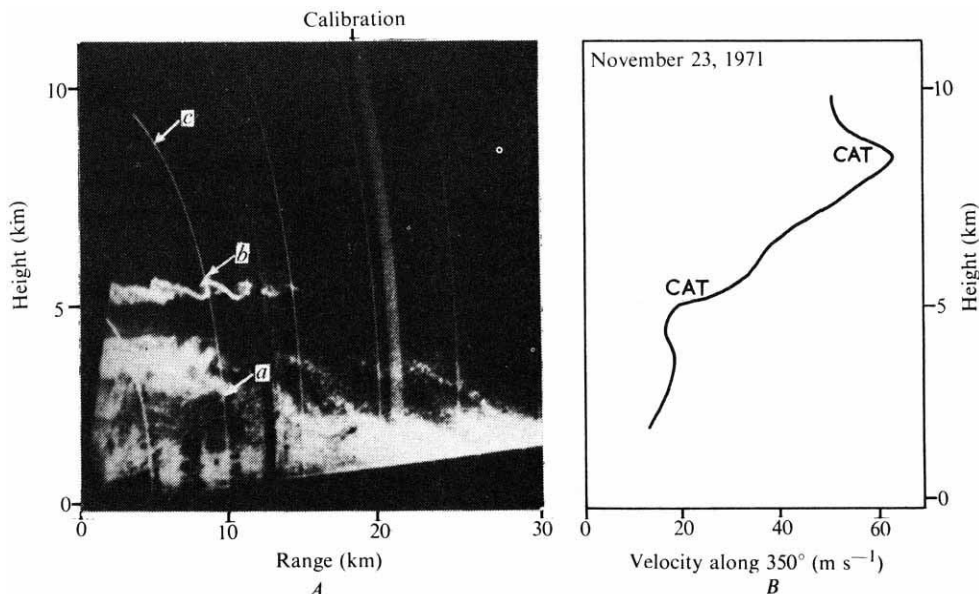
Fig. 2 Histogram of all major primary eruptions from Stromboli since 1899 as a function 25° increments of tidal phase. A 25° phase increment corresponds to approximately 1 day. The standard deviation from the mean is 2.9 day and the 95% confidence interval about the mean is 5.7 day.

strain meters⁹ and gravimeters¹⁷ also gave good agreement for the tidal amplitude, phase and period.

For each of the major Strombolian eruptions (when substantial volumes of material were ejected), the Earth tide was generated for a one-month period beginning 10 day prior to the eruption. Fig. 1 illustrates three typical examples of the relation between variations of the vertical component of the tide and eruptions of Stromboli. The obvious connexion between the eruption times and the modulation envelope derived from the fortnightly tide follows quite consistently throughout the data. Taking all major eruptions from Stromboli between 1900 and 1971, we find the fraction of the total per 25° phase increment of the fortnightly component of the tide as a function of phase (Fig. 2).

Although 67% of the eruptions are known to within an hour, there is no similar clear relation between eruptions and the phase of the diurnal tide. We made a search for correlations with longer tidal periods on the possibility that repose times¹⁸ are tidally controlled although the amplitudes of these longer period tidal components are several orders of magnitude less than the fortnightly and diurnal components. This investigation proved inconclusive.

Fig. 1 A, RHI recording obtained with the Defford radar along azimuth 350° at 1029–1031 GMT on November 23, 1971, showing echo from weak precipitation below 4 km and from the clear air above 5 km. Note the characteristic form of the Kelvin-Helmholtz billows at heights between 5 and 6 km (b). Echoes associated with similar billows near 9 km (c) were very weak and are not reproduced clearly. B, Wind profile, as determined from a radiosonde released at 1026 GMT from Pershore, 8 km NE of Defford, plotted on the same vertical scale as in the RHI photograph. Winds at most levels were approximately northerly and, since the radar was scanning along 350° , it is the component along this direction which has been plotted.



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M. J. S. JOHNSTON
F. J. MAUK

Department of Geology and Mineralogy,
University of Michigan,
Ann Arbor, Michigan 48104

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- ¹ Mauk, F., and Johnston, M. J. S., *Trans. Amer. Geophys. Union*, **53** (in the press).
- ² Knopoff, L., *Bull. Seismic Soc. Amer.*, **54**, 1865 (1964).
- ³ Tamrazjan, G. P., *Akad. Nauk. SSSR Izv. Ser. Geofiz. No. 7*, 840 (1956).
- ⁴ Stacey, F. D., *Geomagnetica*, 109 (Servico Meteorologico Nacional, Lisbon, 1962).
- ⁵ Gougenheim, A., *Compt. Rend.*, **252**, 330 (1961).
- ⁶ Morgan, W. J., Stoner, J. O., and Dicke, R. H., *J. Geophys. Res.*, **66**, 3831 (1961).
- ⁷ Allen, C., St Amand, P., Richter, C., and Nordquist, J., *Bull. Seismic. Soc. Amer.*, **55**, 753 (1965).
- ⁸ Simpson, J. F., *Earth Planet Sci. Lett.*, **2**, 473 (1967).
- ⁹ Ryall, A., Van Wormer, J. D., and Jones, A. E., *Bull. Seismic Soc. Amer.*, **58**, 215 (1968).
- ¹⁰ Fiske, R. S., and Kinoshita, W. T., *Science*, **165**, 341 (1969).
- ¹¹ Swanson, D. A., *Geol. Soc. Amer. (Abs.)*, **4**, 244 (1972).
- ¹² Imbo, G., *Catalog of the Active Volcanoes of the World*, Part 17 (1965).
- ¹³ Giot, *Smithsonian Institution Short Lived Phenomena*, Card No. 1168 (1971).
- ¹⁴ Lo Giudice, A., *Smithsonian Institution Short Lived Phenomena*, Card No. 1196 (1971).
- ¹⁵ Longman, I. M., *J. Geophys. Res.*, **64**, 2351 (1969).
- ¹⁶ Pettit, J. L., *Trans. Amer. Geophys. Union*, **35**, 193 (1954).
- ¹⁷ Melcoir, P. J., *The Earth Tides* (Oxford-Pergamon, 1966).
- ¹⁸ Rittman, A., *Volcanoes and their Activities* (Interscience, 1962).

Measurements of Air Motion in Regions of Clear Air Turbulence using High-power Doppler Radar

It is widely accepted that the primary cause of clear air turbulence (CAT) in the free atmosphere is Kelvin-Helmholtz (K-H) instability¹. This appears as amplifying waves (billows), with amplitudes ranging from one metre to hundreds of metres, which often break into turbulence on a range of smaller scales. The intensity of the turbulence depends on the billow amplitude and on the magnitude of the vertical shear of the wind across their depth before they break.

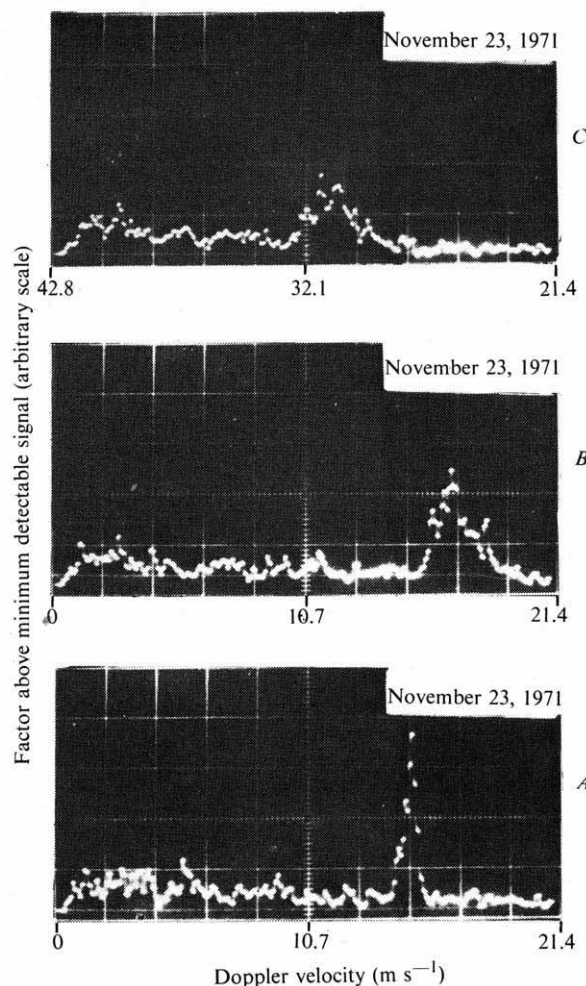


Fig. 2 Doppler velocity spectra obtained with the Defford radar on November 23, 1971, at range 10.1 km at the positions indicated by the arrows in Fig. 1. Spectrum *A* was obtained within precipitation at height 2.7 km. Spectra *B* and *C* were obtained within clear air echoes at heights 5.2 and 8.8 km, respectively. The signal amplitude is displayed on an arbitrary scale, the apparently higher noise level on the left of each display being the result of an equipment defect. The abscissa, representing radial velocity, is labelled 0 to 21.4 m s^{-1} or 42.8 to 21.4 m s^{-1} according to whether the target velocity was less than or greater than the maximum unambiguous velocity of 21.4 m s^{-1} .

Radar detects the incoherent backscatter from inhomogeneities of refractive index resulting from CAT on a scale of half the radar wavelength. In the upper troposphere and above, these inhomogeneities are mainly due to temperature fluctuations, and Ottersten² has argued that the reflectivity of the radar echo is itself a measure of turbulence intensity in such regions. In the lower troposphere, however, humidity fluctuations make a dominant contribution to the inhomogeneities of refractive index so that intense clear air echoes can be produced by relatively weak turbulence. Rain, snow, and even tenuous cirrus clouds also give intense echoes and these can be confused with echoes from CAT. Here, we show by means of a case study that the measurement of the Doppler frequency shift of atmospheric echoes provides information about the wind field which can probably serve as a more reliable indicator of turbulence intensity.

Observations were made on November 23, 1971, while a strong high level jet stream was overhead. We used the Defford (Worcs) radar³, upgraded to give full Doppler velocity information. It was operated to make 0–70° elevation scans into the direction of the high level winds, each up-and-down scan taking 4 min to complete. Data were displayed on a Range Height Indicator (RHI) scope and the bipolar video signals within 10 range gates were recorded on a multitrack

FM tape recorder for subsequent Doppler analysis. The 10 gates, each 100 m wide, were spaced at 200 m intervals between 10 and 12 km.

Fig. 14 shows the RHI display at 1029–1031 GMT. Echoes below 4 km were due to weak precipitation, but according to a special radiosonde released at 1026 GMT, the relative humidity decreased rapidly with height, reaching 40% at 5 km, so that echoes at 5 km and above were from clear air. The echo between 5 and 6 km has the characteristic appearance of K-H billows as reported elsewhere^{3–5}, and the echo between 8 and 10 km, although scarcely visible in this photograph, also has the appearance of K-H billows. The wind profile in Fig. 1*B* shows that the clear air echoes were located within strongly sheared layers below and just above the upper tropospheric wind maximum. The Richardson number at the levels of the billows dropped to just below 0.25.

Fig. 2 shows examples of Doppler spectra obtained by replaying the tape-recorded bipolar video signal through an audio-spectrum analyser. Each spectrum was derived by integrating over a 1 s interval. The aerial was scanned at 0.6 deg s^{-1} so that, at the ranges concerned, spectra were obtained at 100 m height intervals. The dimension of each resolution cell was about 300 m in both range and height. The bandwidth of the clear air echoes was of the order of a tenth of the unambiguous bandwidth of 400 Hz, and the resulting coherent integration gave a ten-fold enhancement of signal-noise ratio. This accounts for the fact that the clear air echo in Fig. 2*C* is easily detectable above noise, whereas it is difficult to discern it in Fig. 1*A*.

Doppler spectra similar to those in Fig. 2 were obtained during a sequence of RHI scans (including the one shown in Fig. 1) and these have been used to derive Fig. 3*A*, *B* and *C*, which shows detailed reconstruction of the lower train of K-H billows as observed along a direction roughly normal to the axis of the billows. Fig. 3*A* shows the pattern of reflectivity. It illustrates the tendency for reflectivity to become large in the largest billows. Fig. 3*B* shows the component of the horizontal wind in the direction of the scan. It is consistent with air of low momentum having ascended in the crests and air of high momentum having descended in the troughs of the billows. An aircraft flying in the section at altitude 5.4 km would have encountered changes in headwind of up to 5 m s^{-1} within a kilometre. The vertical wind shear associated with the billows was chiefly in the range 2 to 7 m s^{-1} (200 m)^{–1}. Knowledge of the magnitude of the shear, when combined with knowledge of the development of large-amplitude K-H billows as depicted on the RHI display, should prove to be sufficient evidence of the presence of significant turbulence. The actual intensity of the turbulence to be expected from any given combination of shear and billow amplitude will have to be determined empirically, however, on the basis of further combined radar-aircraft measurements such as those reported by Browning *et al.*⁶.

The isopleths in Fig. 3*C* depict the distribution of the standard deviation (s.d.) of the Doppler spectrum, σ_v , as derived from the width of spectra such as those in Fig. 2. For clear air echoes, $\sigma_v^2 = \sigma_s^2 + \sigma_t^2$, where σ_s is the s.d. due to the radial component of the wind shear across the depth of the resolution cell and σ_t is the s.d. due to turbulence within the resolution cell. In the study reported here we found σ_v for the clear air echoes to be influenced to a similar extent by small-scale turbulence and by vertical shear; sometimes one dominated, sometimes the other. Thus σ_v is not a direct measure of σ_t . Because strong shear in a region of breaking billows tends to give rise to strong turbulence, however, it is likely that a large value of σ_v can still be taken as a semi-quantitative indication of the intensity of small-scale turbulence.

The most important air motions for the flight safety of large aircraft are those on scales of a few hundred metres. A direct measure of wind fluctuations on these scales is provided by spatial variations in the mean Doppler velocity between one radar resolution cell and another (Fig. 3*B*). Another way of

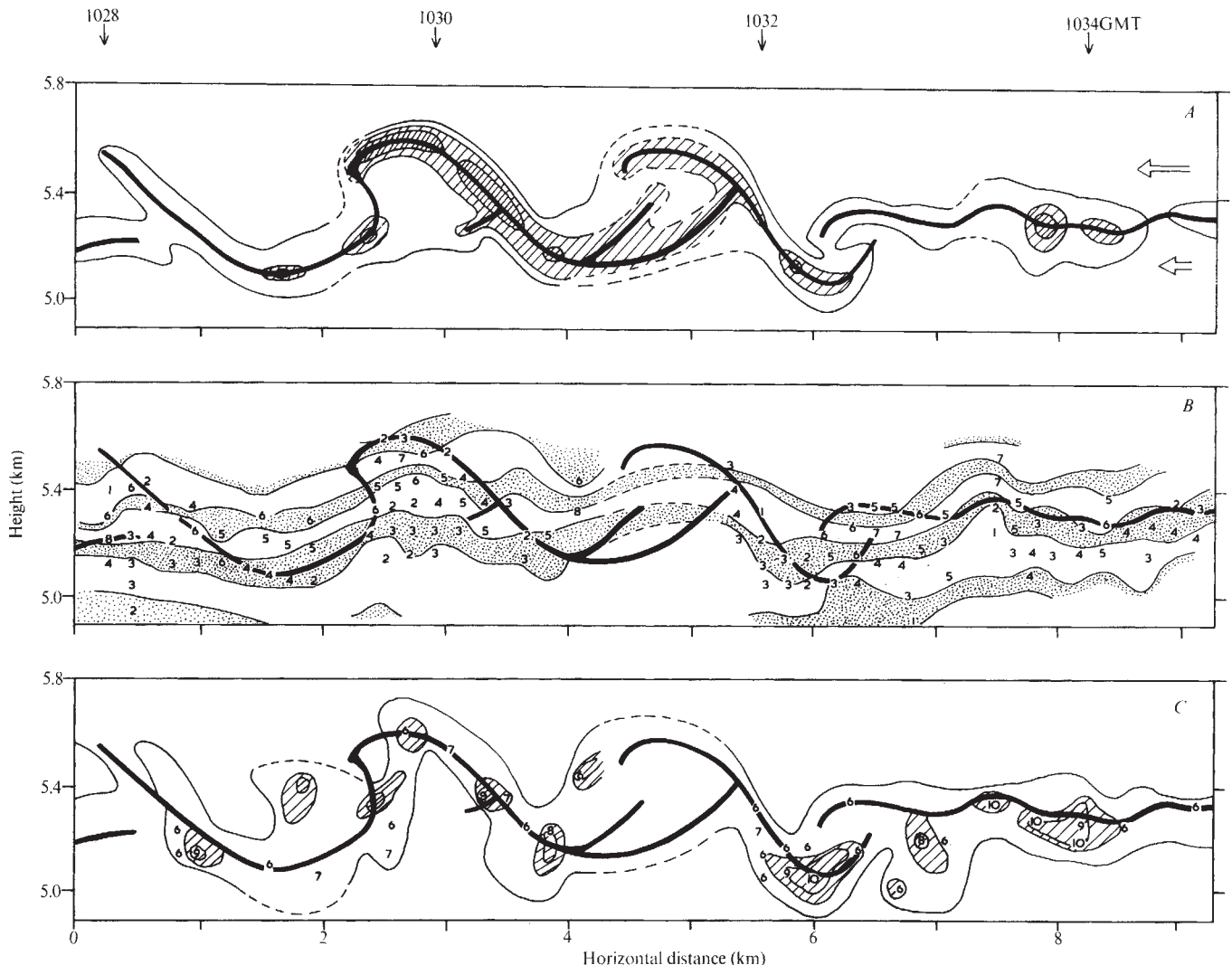


Fig. 3 Vertical sections showing the structure of the train of K-H billows situated at about 5 km altitude, as reconstructed from tape-recorded Doppler radar data within 10 gates at ranges 10 to 12 km during 4 RHI scans (2 upscans and 2 downscans). Successive scans have been displaced by distances according to the 22 m s^{-1} translation velocity of the train of billows. Times at which the corresponding parts of the pattern were at a range of 10 km are indicated at the top of the figure. *A*, Radar reflectivity η , with contours at $\eta=1, 3.5 \times 10^{-14} \text{ m}^{-1}$ (not corrected for incomplete beam filling). Regions with $\eta > 3$ and $5 \times 10^{-14} \text{ m}^{-1}$ are hatched thinly and thickly, respectively. The thick line, also reproduced in *B* and *C*, represents the axis of the most intense echo. *B*, The component of the wind (U) along 350° , derived by neglecting the radial component due to any vertical air motion. Isopleths are at 2 m s^{-1} intervals, with stippled shading for $16\text{--}18 \text{ m s}^{-1}$ (bottom of figure), $20\text{--}22, 24\text{--}26$ and $28\text{--}30 \text{ m s}^{-1}$ (top of figure). Digits represent the corresponding component of vertical wind shear ($\Delta U/\Delta z$) over 200 m height increments, in units of $\text{m s}^{-1} (200 \text{ m})^{-1}$. *C*, The standard deviation of the Doppler spectrum (σ_v), with isopleths at $0.6, 0.8$ and 1.0 m s^{-1} ($> 0.8 \text{ m s}^{-1}$, hatched), and also the estimated standard deviation due to turbulence at scales smaller than the radar resolution cell (σ_t), with digits representing the value of σ_t in units of 10 cm s^{-1} in regions where $\sigma_t \geq 0.6 \text{ m s}^{-1}$.

observing these larger scale wind fluctuations would be to keep the radar beam fixed at a constant elevation and to measure the time changes of mean velocity within each of a large number of range intervals.

K. A. BROWNING
J. R. STARR
A. J. WHYMAN

*Meteorological Research Unit,
Royal Radar Establishment,
Malvern*

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- ¹ Roach, W. T., *Clear Air Turbulence* (Marrow Publishing, 1972).
- ² Ottersten, H., *Proc. Thirteenth Radar Meteorology Conf.*, 252 (Amer. Meteor. Soc., Boston, 1968).
- ³ Browning, K. A., and Watkins, C. D., *Nature*, **227**, 260 (1970).
- ⁴ Hicks, J. J., and Angell, J. K., *J. Appl. Meteor.*, **7**, 114 (1968).
- ⁵ Browning, K. A., *Quart. J. Roy. Met. Soc.*, **97**, 283 (1971).
- ⁶ Browning, K. A., Watkins, C. D., Starr, J. R., and McPherson, A., *Nature*, **228**, 1065 (1970).

Charge Production, Supertankers and Supersonic Aircraft

A RECENT series of explosions aboard supertankers during tank cleaning operations (using high pressure water jets) has raised the possibility that the sudden electrical discharge of an electrostatically charged mist may ignite an explosive mixture of hydrocarbon and air¹. Although the discharge configuration (cloud-electrode) is somewhat different from the usual electrode-electrode situation used to obtain minimum ignition energies, it is to be expected that the discharge energy and hence the volume charge density of the mist will be an important factor in determining the likelihood of an explosion. It is known that a mist is produced during tank cleaning operations and that its charge density stabilizes at a value $\sim 10^{-8} \text{ C m}^{-3}$. The mechanisms responsible both for charging and for then limiting the charge density to such a value are, however, not fully understood².

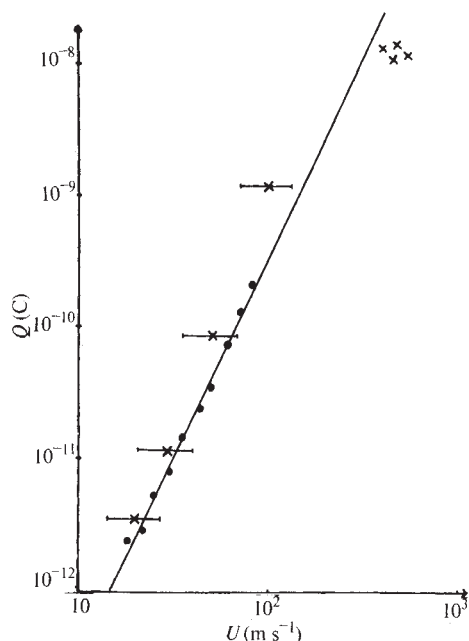


Fig. 1 Charge transfer from a drop by impact and air blast disintegration of a drop as a function of velocity.

A related phenomenon, which is concerned with a quite different range of velocities, is the electrostatic charging of aircraft passing through heavy rain³. Research at subsonic airspeeds has shown that charging currents of up to $\sim 10^{-3}$ A can generate frequencies in the GHz region—and there are the attendant problems of electromagnetic interference⁴—but there is a paucity of information about behaviour at supersonic speeds although Nanevitz and Vance⁵ have shown that for the more likely situation of passage through an ice fog the charging effect eventually falls off with increasing velocity.

In both the situations described, drops of water are very rapidly disintegrated into a cloud of droplets, by impact, by aerodynamic drag, or by both. In this communication, I shall report a series of measurements which extend the range of previous work (Lenard⁶, for example, used velocities less than 20 m s^{-1}) to much higher velocities, 20 to 80 m s^{-1} for impact disintegration and 20 to 500 m s^{-1} for aerodynamic disintegration. Both sets of measurements cover the range of velocities appropriate to tank cleaning operations ($\sim 40 \text{ m s}^{-1}$), whereas the measurements at high velocity are of direct applicability to the problem of the supersonic airliner and do give some information on behaviour when very rapid and complete atomization is achieved.

For the impact measurements, a known and reproducible impact velocity was achieved by releasing a single drop (radius 2 mm) from a capillary and arranging that, as it fell, it collided with a moving aluminium sphere (radius 3 mm) mounted at the end of a rotating arm 15 cm long. The velocity of the sphere was variable up to 80 m s^{-1} and the charge transferred to the sphere as the drops disintegrated (equal and opposite to the charge on the ensuing mist) was measured with an oscilloscope connected to the rotating arm through a pin/mercury commutator on the axis of rotation of the arm. A picoammeter was also used to obtain the average of many disintegrations.

The air blast measurements were carried out using a compressed air nozzle for the subsonic measurements and a shock wave produced in a conventional shock tube for the supersonic measurements. The drop was placed initially upon a conducting plate which, again, was connected to an oscilloscope so that the charge transfer on disintegration, Q , could be measured. The results of both sets of measurements are shown in Fig. 1, and a typical current oscillogram for the air blast

measurements is shown in Fig. 2a. The charge transfers measured at the same velocities by both methods were of similar magnitude, indicating perhaps that the rapidity of disintegration ($\sim 10^{-3} \text{ s}$) was more important than the method by which it was achieved. At subsonic velocities, the charge transfer varies as $\text{velocity}^{3.0}$ in reasonable agreement with the variation as $\text{velocity}^{2.0}$ found by Adkins⁷ at much lower impact velocities. In fact, extrapolation of Fig. 1 towards these lower velocities gives quite good agreement with Adkins's measurements. At the highest velocities, there is a pronounced trend towards "saturation" of the charge transfer.

As the self capacity of an individual droplet is $\propto \text{volume}^{1/3}$ an increase of charge transfer with velocity seems reasonable in view of the natural tendency towards more complete atomization with increasing velocity. For the air blast measurements, this tendency is demonstrated by the relation⁸

$$U^2 d \approx 6 \times 10^5 \text{ c.g.s. units} \quad (1)$$

where d is the maximum stable diameter of a drop in an air-flow of relative velocity U . For the highest velocities, a simple space charge calculation shows that with $Q \sim 10^{-8} \text{ C}$ and $r = 2 \times 10^{-1} \text{ cm}$, and with charged droplets leaving the disintegrating drop at a velocity no greater than the flow velocity, the electric field of the space charge just downstream of the drop will be $\sim 10^6 \text{ V m}^{-1}$ or more. (The charged droplets may well be travelling at a speed considerably less than the flow velocity.) Under these conditions gas breakdown is clearly likely and, if it occurs, it will act to reduce or limit the charge transfer.

Examination of the disintegrating drop with a photomultiplier revealed that, at the highest velocities, light was emitted (an oscillogram is shown in Fig. 2b). At lower velocities, no emission was observed and so it is concluded that gas breakdown due to the electric field of the space charge is probably responsible for the observed "saturation" in charge transfer with increasing velocity. These results may be of some significance to supersonic flight, for they indicate first, that the charging current to the aircraft may be about an order of magnitude greater than at subsonic speeds and, second, that ionization may be generated by electrical breakdown and hence cause further electrical noise problems.

Although the measurements at low velocities (Fig. 1) can be used to predict an impact charging rate for droplets of appropriate radius that disintegrate during tank cleaning operations, they do not yield any information about a more

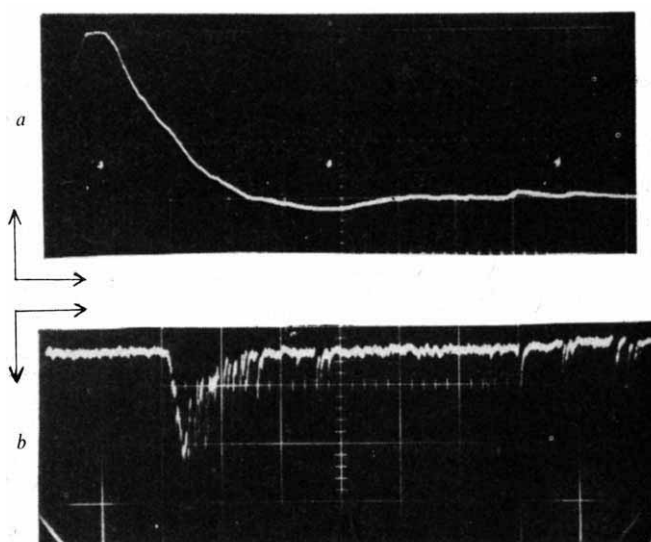


Fig. 2 a, Charging current for subsonic impact as a function of time. (Current = 2 nA/division ; time = 1.0 m s/division ; velocity $\approx 20 \text{ m s}^{-1}$); b, light output from a disintegrating drop in supersonic air flow as a function of time (0.2 m s/division).

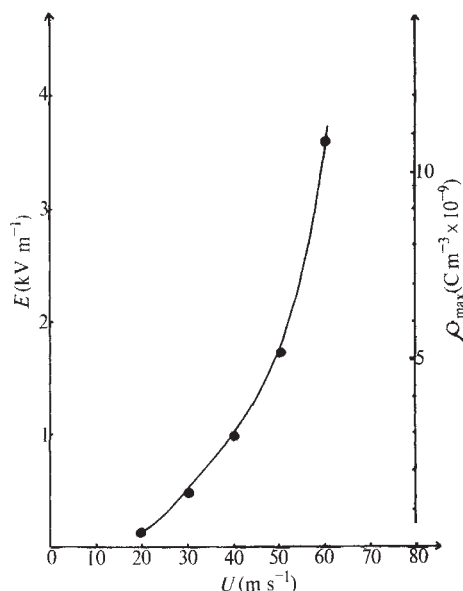


Fig. 3 ●, Critical electric field for zero net charge transfer as a function of impact velocity; —, maximum charge density computed for supertanker dimensions as a function of impact velocity.

significant quantity—the steady state charge density of the mist in the tank. One possible limitation, again arising from space charge electric fields, was investigated in some detail. For a spherical tank of radius r_0 containing mist of charge density ρ , the electric field at the wall is

$$E \sim \frac{\rho r}{3\epsilon_0} \quad (2)$$

As ρ increases as a consequence of the impact of drops from a hose, so will the charge induced by E upon each disintegrating droplet⁷. This induced charge is of opposite sign to the charge produced on impact and thus, at a given electric field, the net rate of charge production will fall to zero. Such a limit for a continuous jet of water has been explored by Vos⁹. The critical electric field appropriate to a single drop of radius 2 mm was measured as a function of velocity by using the rotating sphere described previously. In this case, the sphere was biased to such a voltage that the net charge per disintegration was zero, and the critical field was then calculated from the known radius of the sphere. The results are shown in Fig. 3; at 50 m s⁻¹ the limiting space charge field $\sim 2 \times 10^3$ V m⁻¹. More significant, from the point of view of supertankers, is the curve in Fig. 3 which is based on calculations of the charge density in a tank of radius 10 m (the supertanker situation) from the measured critical electric field using equation (2); the limiting charge density is very close to that observed in practice. The results of Fig. 3 are, however, only directly applicable to the supertanker situation if all charged droplets remain suspended in the air. Lenard and others have shown that the larger, less buoyant droplets tend to carry positive charge, and the lighter droplets negative charge. Thus the limit derived above may in practice be slightly less severe. But the fact that this limit is so close to that observed in practice indicates that charging by impact disintegration of drops followed by space charge limitation of charge density may well be the mechanisms pertinent to conditions in a supertanker.

The situations considered here pertain to very different ranges of velocity. Measurements with single drops, however, show a reasonably continuous variation from one regime to the other, and additional evidence indicates that space charge limiting mechanisms apply to both cases.

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P. R. SMY

Department of Electrical Engineering,
University of Alberta,
Edmonton,
Alberta

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- ¹ van der Meer, D., *Proc. Third Conf. Static Electrification* (London, 1971).
- ² Hughes, J. F., *Nature*, **235**, 381 (1972).
- ³ Odam, G. A. M., *Proc. First Intern. Conf. Static Electricity* (Vienna, 1970).
- ⁴ Truax, R. L., *Proc. First Internat. Conf. Static Electricity* (Vienna, 1970).
- ⁵ Nanevich, J. E., and Vance, E. F., *IEEE Trans. Aerospace and Electronic Systems*, **AES 3**, 628 (November 1967).
- ⁶ Lenard, P., *Ann. Phys.*, **46**, 584 (1892).
- ⁷ Adkins, C. J., *Quart. J. Meteorology Soc.*, **85**, 237 (1959).
- ⁸ Lane, W. R., *Ind. Eng. Chem.*, **43**, 1312 (1951).
- ⁹ Vos, B., *Proc. Third Conf. Static Electrification* (London, 1971).

Paired Metamorphic Belts of the Gondwanaland Precambrian and Plate Tectonics

RECONSTRUCTIONS of Gondwanaland, based on geological arguments, paleomagnetic data and best fit of continents have derived two possible models of reassembly¹⁻⁵. The most popular places India-Ceylon adjacent to East Antarctica¹; the other places it next to Western Australia⁴. The exact position of Madagascar in these models has been the subject of some debate, but it is now placed north of its present position against East Africa⁶. Both models have reasonable geological correlations, but a rigorous test of fits based on Precambrian basement correlations has been attempted in a limited way only between India-Ceylon, Western Australia and East Antarctica^{7,8}. Tracing out dispersed Precambrian belts to test continental drift has also been applied to fits between Brazil and Gabon⁹. I have shown that Precambrian granulite facies, paired metamorphic belts can be traced throughout the basement rocks of Gondwanaland. The significance of these paired belts in terms of plate tectonics during the Precambrian is also discussed.

The paired metamorphic belts of Japan and the circum-Pacific area^{10,11} are characterized by an outer-ocean side high-pressure belt of the Sanbagawa type and an inner high-temperature, low-pressure belt of the Abukuma type. Most of these paired belts were formed in Mesozoic times, although occurrences in Scotland and other localities suggest incipient formation in the Palaeozoic. Studies on the facies series of the Precambrian granulite facies basement rocks of Gondwanaland centred on Ceylon-South India^{12,13} indicate paired metamorphic belts of Precambrian age.

Consistent elements in the make up of the Gondwanaland Precambrian basement are rocks of the granulite facies or "charnockites"¹⁴. These rocks can be traced from Ceylon-India through Madagascar and Uganda to the west and to East Antarctica and South-west Australia to the east. The belts can be divided into two units that run side by side and form a paired metamorphic "Gondwana Granulite Belt" displaying a gross metamorphic, structural and chronological unity, characterized by highly deformed, granulite facies rocks of Archaean age (about 3,000 m.y.). These rocks have been subjected to periods of metamorphism and/or remobilization at about 2,000 m.y., 1,300 m.y., and 1,000 m.y., ending with a pervasive metamorphic event at about 600 m.y. which recrystallized all the rocks and formed the paired metamorphic characteristics¹³. This last episode, known as the Indian Ocean Cycle¹⁵, is reflected in the widespread K-Ar mineral dates common to all Gondwana basement units.

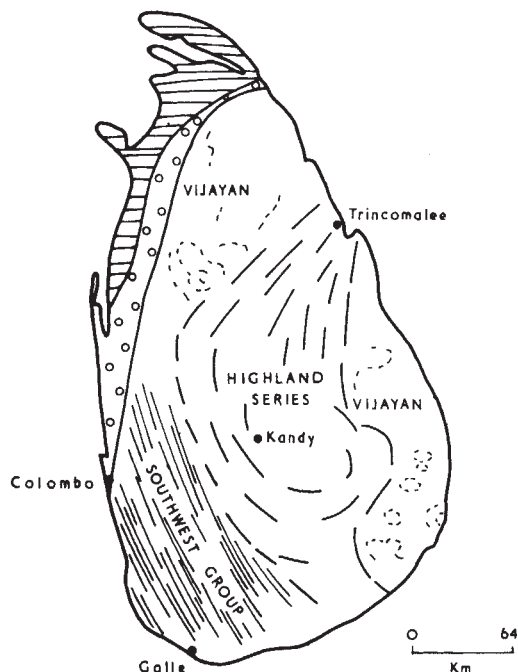


Fig. 1 Geology of Ceylon showing the Highland Series-Barrovia, South-west Group-Abukuma, paired metamorphic belt and the Vijayan basement. Linear patterns represent general structural trends. Horizontal lines, Miocene limestones; stippled region, recent coastal sediments.

Studies on the Precambrian granulite facies of Ceylon¹² revealed that these rocks can be divided into two contrasted facies series; the Highland-Barrovia type facies series and the South-west-Abukuma type facies series (Fig. 1).

The Highland rocks are characterized by intermediate pressure-temperature mineral assemblages with sillimanite in meta-pelites, diopside-scapolite calciphyres and forsterite-clinohumite magnesian marbles. The South-west rocks, however, contain cordierite (sometimes with sapphirine) in rocks of appropriate composition, andalusite locally coexisting with sillimanite in metapelites and wollastonite in calciphyres, which place these rocks in a high temperature category. These terrains run side by side, are of the same age and together constitute a paired metamorphic belt¹³. On models of reconstructed Gondwanaland this paired belt can be traced into Southern India, Madagascar, Uganda and into East Antarctica and South-west Australia.

The origin of paired metamorphic belts of Japan, has been related to the arc-trench system of the Japanese Islands¹¹. The trench lies in a zone of high pressures and low temperatures, and the volcanic arc in a zone of high temperatures and low pressures. These two contrasted metamorphic environments produce high pressure-Sanbagawa and high temperature-Abukuma types of facies series respectively. The arc-trench system, in terms of plate tectonics, reflects interaction between a downward descending oceanic plate and a rather static continental plate. The zone of subduction of the trench is the region of high pressure metamorphism and the volcanic arc is the surface expression of the inclined seismic zone where a high temperature metamorphism is manifest¹⁶. Thus the metamorphic reflexion of this plate tectonic model of the arc-trench system is a paired metamorphic belt with an outer high pressure metamorphism of the trench and an inner high temperature metamorphism of the arc. The paired arrangement of the Sanbagawa-Abukuma type is characteristic of the younger Mesozoic metamorphic belts of the circum-Pacific area. Recognition of older paired belts may be evidence for Pre-Mesozoic plate tectonics.

The paired metamorphic belts of the Barrovia-Abukuma type in the granulite facies rocks of Gondwanaland suggest Precambrian plate tectonics. This belt was formed at depth

and conceivably had the normal Sanbagawa-Abukuma equivalents near surface. The seeming absence of high pressure belts in older Precambrian rocks have been discussed¹⁰. Late Precambrian-lower Paleozoic high pressure belts have, however, been reported from the Urals, West Sayan and Kazakhstan in the USSR¹⁷. Special tectonic conditions and movement rates along the subduction zone will determine whether or not these high pressure belts are preserved¹⁸. The Gondwana belts under discussion were formed at depth under granulite facies and an examination of PT facies series diagrams will show that under these conditions (600°-800° C, -4-7 kbar), high pressure Sanbagawa types of metamorphism are not realized (Fig. 2). It is considered that the Barrovia belt reflects an ancient trench subduction zone and it is the granulite facies equivalent of the supracrustal Sanbagawa facies series. The complementary Abukuma belt is of arc type and together with the Barrovia belt constitute a metamorphic pair, reflecting an arc-trench system formed by plate tectonic interaction.

The Gondwana metamorphic belts are traced through both models of reconstructed Gondwanaland. In one model^{4,5,13}, this paired belt can be followed in a linear path from Uganda through Madagascar into Ceylon-South India (Fig. 3). The extensions into south-western Australia can be traced into the Wheat Belt and further extrapolations are possible for Eastern Antarctica, but these are not clear. It also appears that a Barrovia belt forks off from the Uganda-Madagascar-Ceylon-South India trend and follows the Eastern Ghats, but this belt has no recognizable Abukuma equivalents. A more rational

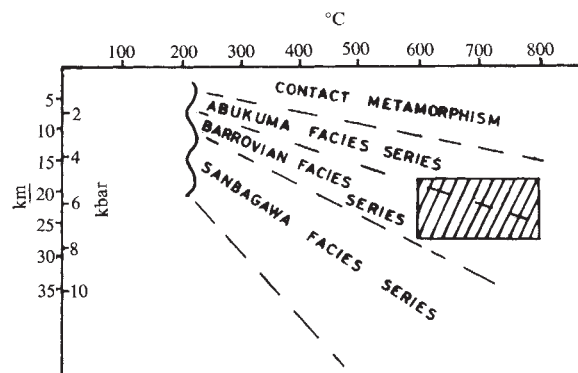


Fig. 2 Schematic PT diagram for different types of facies series. Shaded area delineates conditions of the granulite facies series which only occur in the Abukuma and Barrovia facies series.

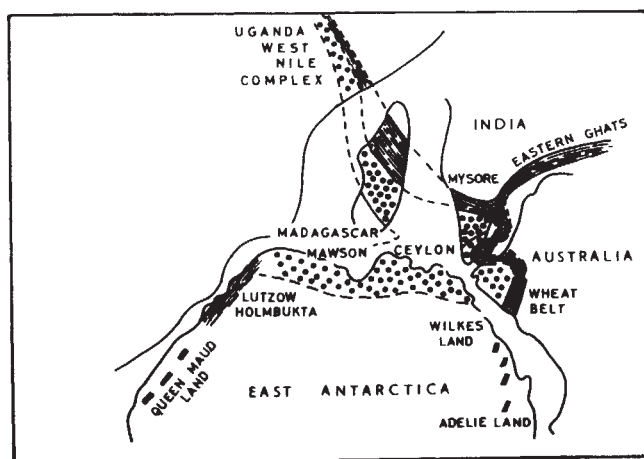


Fig. 3 The Precambrian paired metamorphic belts as plotted on one model of Gondwanaland^{4,5}. Linear belt represents Barrovia-Intermediate P/T facies series. Stippled belt represents Abukuma-Low P/High T facies series. Solid belt boundaries are actual geological correlations and dashed belt boundaries are inferred. Bold bar patterns represent similar metamorphic belts which are not well defined and whose relationship to the scheme is uncertain.

pattern emerges if we are to plot these metamorphic belts onto the more popular model of reconstructed Gondwanaland¹⁻³ (Fig. 4). The linear belt of Uganda–Madagascar–Ceylon–South India is still intact, but in the vicinity of Ceylon and adjacent south-eastern India the trend seems to fork to form a Y-shaped pattern. One fork follows the Eastern Ghats of Barrovian-type, but in this model an Abukuma complement appears in eastern Antarctica centred around Mawson¹⁹. This belt may continue on to join up with similar rocks found in the Wheat Belt of south-western Australia²⁰. The other fork trends towards Queen Maud Land of Eastern Antarctica and appears to be mainly Barrovian in nature. The Highland Series of Ceylon together with the Lutzow Holmbukta rocks of East Antarctica²¹ form the beginnings of this belt.

If we are to accept this model as being a more rational reconstruction of Gondwanaland, the paired metamorphic belts should reflect plate tectonic interaction. In a generalized scheme of these belts a pattern emerges which suggests plate tectonics between at least three early Archaen fossil proto-plates which formed about 3,200 m.y. ago (Fig. 5). These fossil proto-plates consist of unmetamorphosed low grade sedimentary and volcanic rocks which make up the greenstone belts of the Archaen cratons²². The Indian proto-plate,

represented by the Dharwar²³, faces the outer Barrovian belt and is thought to be of oceanic type. Both the African proto-plate, represented by the Rhodesian–Transvaal block, and the Antarctic–Australian proto-plate, represented by the Yilgarn block are adjacent to the inner Abukuma belt and are thought to be of continental type. Separating these two continental proto-plates is a Barrovian belt which does not show paired relationships. If the extrapolation of this belt into South Africa to join with the Limpopo belt is valid, the Transvaal block of the African proto-plate may actually be part of the Antarctic–Australian plate.

This pattern is interpreted in light of plate tectonics as a Precambrian interaction between an oceanic Indian proto-plate and two continental proto-plates which may or may not have been joined at the time. The Indian plate subducted under the African and Antarctic–Australian plates to form a trench-arc system which is now reflected in the paired metamorphic belts; the Barrovian portion represents the trench environment and the Abukuma part represents the arc environment, at the equivalent depth of the granulite facies. The Barrovian belt separating the two continental proto-plates is interpreted as representing the catazonal juncture of two plate collisions.

These Precambrian paired metamorphic belts of granulite facies type are thus the high-grade equivalents of the low-grade Archaen cratonic greenstone belts and their patterns delineate Precambrian plate tectonic interactions. Similar Precambrian high grade metamorphic belts, whether paired or not, may have been formed in a similar manner.

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MICHAEL B. KATZ

*School of Applied Geology,
University of New South Wales,
PO Box 1 Kensington,
New South Wales*

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- ¹ Smith, A. G., and Hallam, A., *Nature*, **225**, 139 (1970).
- ² McElhinny, M. W., *Nature*, **228**, 977 (1970).
- ³ Dietz, R. A., and Holden, J. C., *J. Geophys. Res.*, **75**, 4939 (1970).
- ⁴ Tarling, D. H., *Nature*, **229**, 17 (1971).
- ⁵ Veevers, J. J., Jones, J. G., and Talent, J. A., *Nature*, **229**, 383 (1971).
- ⁶ Sowerbutts, W. T. C., *Nature*, **235**, 435 (1972).
- ⁷ Crawford, A. R., *Nature*, **223**, 380 (1969).
- ⁸ Crawford, A. R., *J. Geol. Soc. India*, **12**, 205 (1971).
- ⁹ Allard, G. O., and Hurst, V. J., *Science*, **163**, 528 (1969).
- ¹⁰ Miyashiro, A., *J. Petrology*, **2**, 277 (1961).
- ¹¹ Miyashiro, A., *Dansk. Geol. For.*, **17**, 390 (1967).
- ¹² Katz, M. B., *Geol. Rundsch.*, **60**, 1523 (1971).
- ¹³ Katz, M. B., *Int. Geol. Congr. Twenty-fourth Sess., Montreal*, Sect. 2, 43 (1972).
- ¹⁴ Hurley, P. M., and Rand, J. R., *Science*, **164**, 1229 (1969).
- ¹⁵ Holmes, A., *Proc. Geol. Assoc. Canada*, **7**, 81 (1955).
- ¹⁶ Dewey, J. F., and Bird, J. M., *J. Geophys. Res.*, **75**, 2625 (1970).
- ¹⁷ Sobolev, V. S., Dobretsov, N. L., Reverdatto, U. U., Sobolev, N. V., Ushakov, E. N., and Khlestov, V. V., *Dansk Geol. For.*, **17**, 458 (1967).
- ¹⁸ Oxburgh, E. R., and Turcotte, D. L., *J. Geophys. Res.*, **76**, 1315 (1971).
- ¹⁹ Segnit, E. R., *Min. Mag.*, **31**, 690 (1957).
- ²⁰ Wilson, A. F., *Spec. Publ., Geol. Soc. Austr.*, **2**, 243 (1969).
- ²¹ Suwa, K., *Int. Geol. Congr., Twenty-third Session, Prague*, **4**, 171 (1968).
- ²² Windley, B. F., and Bridgwater, D., *Spec. Publ. Geol. Soc. Austr.*, **3**, 33 (1971).
- ²³ Pichamuthu, C. S., in *The Precambrian* (edit. by Rankama, K.), **3**, 1 (Interscience, New York, 1967).

Three-Dimensional Imaging of Ultrasound: Direct Ultrasonic Visualization of Defects

WHEN a pair of identical converging optical lenses are assembled coaxially with their foci coincident, a three-dimensional object field produces a three-dimensional real image field of the same

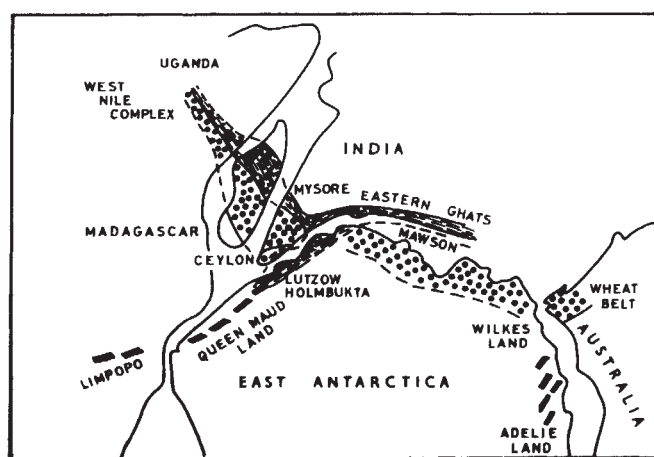


Fig. 4 The Precambrian paired metamorphic belts of reconstructed Gondwanaland¹. Details as for Fig. 3.

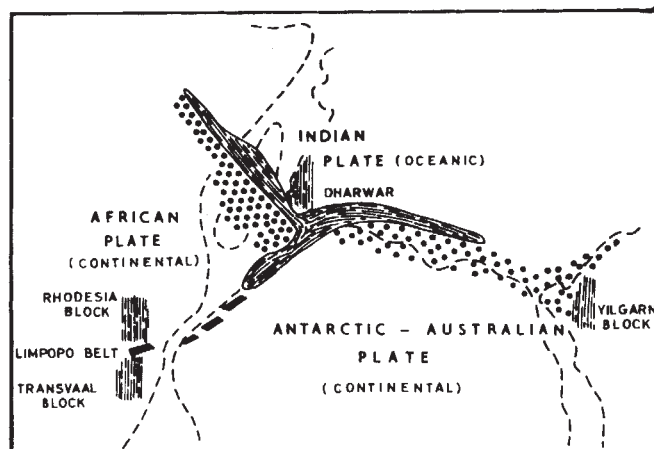


Fig. 5 Schematic diagram showing the relationships between the Precambrian paired metamorphic belts of Gondwanaland and the proto-plates that these belts delineate. Striped pattern represents the Indian proto-plate (Dharwar), the African proto-plate (Rhodesian–Transvaal Block) and the Australian–Antarctic proto-plate (Yilgarn Block). Linear belt is of Barrovian-type and stippled of Abukuma-type. This pattern suggests that the Indian oceanic proto-plate subducted under the continental proto-plates of Antarctic–Australia and Africa to form at depth the paired arrangement.

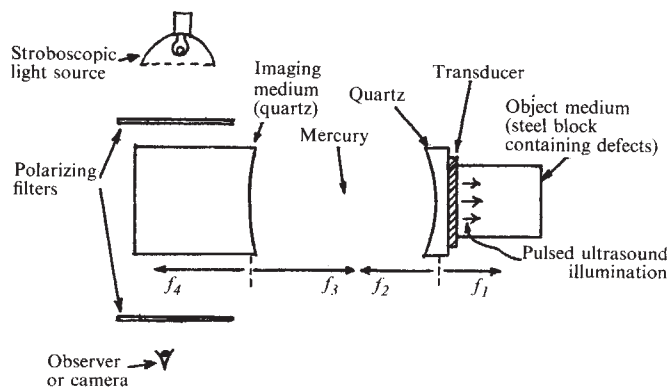


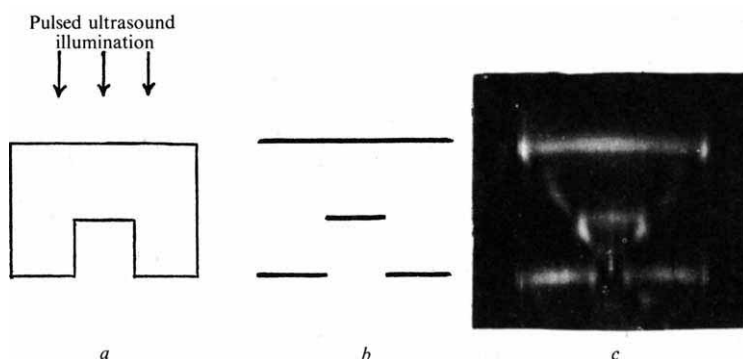
Fig. 1 Ultrasonic focusing and visualizing system.

tuting a thin lens for the relevant curved interface. Computer-aided ray plotting has shown that the required properties can be displayed over a useful transverse field and that aberrations may be controlled by methods analogous to those used in optics.

Using these principles, I have demonstrated direct ultrasonic visualization of defects. For one such demonstration the configuration shown in Fig. 1 was used, the reflecting objects being the faces formed by milling a slot in the steel block (Fig. 2a). Fig. 2c illustrates the ultrasonically reflecting surfaces correctly imaged. Certain spurious bright areas are apparent, but the cause of these is known; development is now aimed at removing them and improving the sensitivity of the method.

Direct ultrasonic visualization of defects offers the prospect of presenting a "real time" display of defects in their correct

Fig. 2 Demonstration of direct ultrasonic visualization of defects. a, The object, a shaped steel block; b, the image expected; c, the image obtained.



shape. The field boundaries in the longitudinal direction are determined by the focal length but in the transverse directions the limits are set only by aberrations and vignetting.

This principle has not previously been applied to ultrasonic focusing. In Fig. 1, I show an arrangement designed to focus ultrasound; two curved interfaces between quartz and mercury replace the two lenses used in the optical system. The transducer "illuminates" the steel block, and any ultrasonically reflecting surfaces, such as defects in the steel, produce reflexions which pass back through the transducer and are focused in the imaging medium. The images may be examined by using any of the established methods for visualizing ultrasound, the most suitable ones being the schlieren method¹ and photoelasticity²; the latter method is used in the arrangement of Fig. 1.

By using ultrasonic illumination in the form of short repetitive pulses, and "freezing" the images by viewing them with stroboscopic light flashes suitably synchronized, ultrasonically reflecting surfaces can be made to produce corresponding bright images, to give direct ultrasonic visualization of defects.

For the system to work, two focusing properties are required: one is the feature referred to above whereby the image field has the same general shape as the object field; this property I term "linearity". The other is that rays from reflecting objects in different parts of the three-dimensional object field must come to their respective foci simultaneously, otherwise a change in timing of the stroboscopic light flashes is required in order to examine defects lying at different depths; I call this property "isochronicity".

Analysis of focusing performance shows that linearity and isochronicity can be incorporated in a two-lens system, such as that in Fig. 1, by imposing two constraints; first, for linearity, the nearer focal points (represented by f_2 and f_3) must be coincident; second, for isochronicity, the further focal lengths must be related by: $f_4 = \sqrt{2} f_1$.

It can be shown that these constraints may be met simultaneously for any set of different sonic velocities in the media concerned (although the velocities affect the object-to-image scaling and the longitudinal field limits); they can also be met when adjacent media have the same sonic velocity, by substi-

spatial relationships, simultaneously imaged, even if they lie at different depths in the material under inspection. This is achieved with access to one side only of the object being examined and without the need for coherent light sources. Although the concept has so far been directed towards engineering applications, it has interesting possibilities in the medical sphere.

P. D. HANSTEAD

Central Electricity Generating Board,
South Western Region,
Scientific Services Department,
Portishead, Bristol

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¹ Burton, C. J., and Barnes, R. B., *J. Appl. Phys.*, **20**, 462 (1949).

² Hui Pih and Snyders, L. S., *Experimental Mechanics*, **9** (4), 186 (1969).

BIOLOGICAL SCIENCES

Numerology of Development

IN chimaeras (for example, embryo-fusion chimaeras in mice) or in females heterozygous for *X*-linked markers (according to the single-active-*X* hypothesis), two genetically distinct cell populations co-exist in the same individual. For characters which are cell-autonomous, so that the genotype of individual cells can be deduced by observing the cell itself (for example, the pigmented layer of the retina) or a cell product (for example, pigment in hairs; electrophoretic enzyme variants), one can estimate the proportion of cells belonging to each population in any given organ or tissue.

From these proportions, inferences are sometimes drawn as to the developmental history of the organ, assuming that both cell populations proliferate at the same rate, that is that there is no cell selection. This assumption is more likely to be valid in the case of heterozygotes for *X*-linked markers, where the two

populations differ with respect to their *X*-chromosome only, than in the case of chimaeras between different strains. The existence of cell selection in chimaeras has been amply demonstrated¹. In what follows, however, I shall ignore the effects of selection.

In the simplest case we can consider the proportion of individuals in which an organ is derived from one or other cell population only. If this proportion is, for example,

$$\frac{1}{16} \left(= 2 \times \frac{1}{2^5} \right),$$

we may deduce that at some point in development

five precursor cells were independently drawn from a random mixture of the two cell populations. The probability of all five being of the same genotype would then clearly be $\frac{1}{16}$. It is from this sort of argument that Mintz² derives figures of three cells for the progenitor cells of the embryo, and two cells for the liver, while Tettenborn, Dofuku and Ohno³ deduce that the kidney proximal tubule cells are "the descendants of only four or five embryonic progenitor cells". Strictly, the argument is only valid if the proportions of organs showing various intermediate amounts of the two cell populations follow a binomial distribution. If (as in the case of the hair follicle mosaicism⁴ mentioned below) the mosaic compositions are not so distributed, the simple binomial argument cannot be used. In practice, however, this information is rarely available.

Can we infer anything about the stage of development for which the population numbers so derived apply? Tettenborn *et al.* assume that it is the stage at which the embryonic cells are "committed to serve as the progenitors" of kidney proximal tubule cells. Mintz regards it as the stage at which the tissue "first becomes differentiated", or at which cells are "set aside" for the development of the tissue, or at which tissue-specific genes are first switched on.

The development of a tissue includes several landmarks which do not necessarily coincide. (1) The first moment when a tissue-specific gene locus is switched on, that is a locus which functions in this tissue only. Additional tissue-specific loci may, of course, be switched on later. (2) The beginning of biochemical differentiation, that is the time when the tissue precursor cells are biochemically distinguishable from their neighbours. This may coincide with (1), but could in principle precede it, if for example a shift occurred in the cytoplasmic translation pattern or ionic composition before gene action was affected, or could follow it if messenger RNA molecules exist in "masked" form. (3) The beginning of morphological differentiation. This is generally assumed to occur later than, and as a result of, biochemical differentiation. (4) Irreversible biochemical differentiation, in the sense that the cells have become incapable of developing along any other pathway, even if their environment is altered by, for example, transplantation to a different part of the body. This can only be investigated by experimental interference with development. It cannot occur earlier than (2) and may occur much later. The concept of irreversible differentiation or commitment is an interesting one which deserves investigation by the experimental embryologist, and analysis by the biochemist, but it is irrelevant to the present question. (5) The onset of clonal development, the time when cell mixing between those cell lineages whose descendants will form part of the tissue and other cell lineages becomes minimal. "Going clonal" could coincide with biochemical differentiation or could precede it, but it seems unlikely that the onset of biochemical differentiation would ever precede the initiation of clonal development. Cessation of cell mixing presumably reflects biochemical differentiation, decreasing cell motility or increasing stickiness; this may involve differentiation of one particular tissue, or may affect a larger area.

Terminology is confused. "Determination" has been variously applied to (2) and (4), and "commitment" to (1), (2) and (5). We suggest below that (5) is the critical stage in the present context.

In considering cell lineages, two statements must be distinguished. At any cell generation between the fertilized egg

and the adult, it is possible to state: (a) "There are *x* cells, which contribute some descendants to the given organ"; (b) "There are *y* cells, which contribute all of their descendants to the given organ".

Of these, (b) is often selected (though seldom explicitly) as implying commitment, that is a population of cells is "committed" to form an organ if all its descendants end up in that organ. (In this sense, there may never be a committed population of cells, for *y* may be zero for all cell generations.) On the other hand, the (b) sub-class of (a) is irrelevant if, as at present, we are concerned with whether two types of cell or only one are ultimately represented in the organ. If some members of a clone go to form the parathyroid rather than the thyroid, it will not affect which populations are represented in the thyroid.

It is statement (a) that is relevant to the representation of cell types in an organ. For each cell generation, there must exist a non-zero value of *x*. For the one-celled zygote, *x*=1. In considering the contribution of two different cell populations to an organ, we are concerned only with cell generations at and after the point *h* at which inter-cellular genetic heterogeneity arose, that is for fusion chimaeras at the eight- to sixteen-cell stage (two eight-cell embryos are normally fused to give a sixteen-cell aggregate, though fusion both earlier and later in cleavage is possible) and for *X*-inactivation mosaics at some undetermined point or points subsequent to blastocyst formation^{5,6}.

In theory we could plot *x* against cell generation from *h* up to adulthood. If each of the original *x_h* cells proliferated as a discrete clone, with little cell mixing, the value of *x* would increase at each cell generation (it would not necessarily double, because not all the cells in each clone end up in the same organ). At the other extreme, if thorough cell mixing occurred, so that the descendants of the original *x_h* cells were randomly distributed, the value of *x* would remain constant, or nearly so, once the total number of cells in the embryo became large relative to *x*.

In practice, the later cell generations leading up to the adult organ are likely to be clonal, with any cell mixing occurring within the organ only, while at an earlier stage free mixing between *x* and non-*x* cells may occur. If mixing has been thorough, the two genetically distinct cell populations will be randomly distributed at the point when clonal development begins to predominate (listed as (5) above). The value of *x* at this point will therefore coincide with the estimate *x_B* derived by binomial considerations from the proportion of unmixed organs. For earlier cell generations, the value of *x* will remain constant so long as mixing is thorough and the total number of cells (*N*) in the embryos is large relative to *x*. Once *N* becomes small relative to *x*, binomial sampling theory no longer applies. Even with thorough mixing, some of the *x* cells will share parents, so that *x_h* will be less than *x_B*.

Thus we can conclude that the binomially derived estimate of "four or five embryonic progenitor cells" for the kidney proximal tubules applies to the cell generation at which clonal development of the tubules is initiated, if this occurs later than *X*-inactivation; and that the same estimate holds for the cell generation at which *X*-inactivation occurs, provided that the total number of cells in the embryo is fairly large at that time. If it is small, then the number which will contribute at least some descendants to the tubules will be less than four or five.

If the two genetically distinct cell populations are not randomly distributed at the time that clonal development of the organ begins, that is if some "macro-cloning" has preceded "micro-cloning", the value of *x* at this time will not coincide with the binomially derived *x_B*. That such a situation is biologically plausible is seen from studies⁴ on the mosaic composition of individual hair follicles in women heterozygous for an *X*-linked marker. The composition of the follicles did not follow a binomial distribution, but showed a good fit to a model in which small groups of four to six cells are sampled from a mosaic tissue where the two cell types are already arranged in larger clones of twelve to sixty cells.

Nesbitt⁷ has analysed the mosaicism resulting from *X*-chromosome inactivation in various ectodermal and mesodermal tissues of mice heterozygous for a cytologically detectable *X*-chromosome translocation, using a more elaborate statistical approach which takes into account the covariance of different tissues. Differences between mice with respect to the mean level of mosaicism for the tissues examined (that is covariance within individuals) suggest that a random sample of about twenty-one cells is common to the ancestry of all the tissues. Nesbitt argues that this sampling event is to be equated with the occurrence of *X*-chromosome inactivation. The total number of embryonic cells at the time of inactivation could be considerably greater than twenty-one if not all the cells contribute to the tissues examined and in particular if ectoderm-endoderm divergence has already occurred. From the independent variation of the different tissues within individuals, she deduces that a further sampling event involving twenty to fifty cells is unique to the cell lineages of each tissue type, and speculates (without further definition) that this event consists of "determination of the primordial precursor cells for the tissue". This concept, which relates to the distribution of embryonic cells to different organs, may be expressed as a statement additional to (a) and (b) above, namely (c) "There are z cells, which contribute some descendants to organ M and none to organ N ". At each cell generation, z will get larger; presumably by the "determination of the primordial precursor cells for the tissue", Nesbitt means the first cell generation at which z is greater than zero.

In summary, the estimate x_B of the number of "progenitor" cells for an organ, derived by application of the binomial theorem (on certain assumptions) to the proportion of genetically homogeneous organs in genetically heterogeneous individuals, can in the absence of independent evidence be applied only to the cell generation at which clonal development is initiated. If development is clonal throughout, that is there is no phase of cell mixing, x_B will apply only to the stage at which heterogeneity arises. There is no *a priori* reason why the onset of clonal development should coincide with biochemical or morphological differentiation, whether reversible or irreversible, or with the activation of organ-specific genes. The same estimate x_B will hold for earlier stages of development, including the stage at which heterogeneity arises, provided that the total number of cells in the embryo is large relative to x_B , and that effective mixing takes place.

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ANNE McLAREN

ARC Unit of Animal Genetics,
Institute of Animal Genetics,
King's Buildings,
West Mains Road,
Edinburgh 9

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¹ Mintz, B., in *Genetic Concepts and Neoplasia*, 477 (Williams and Wilkins, Baltimore, 1970).

² Mintz, B., in *Control Mechanisms of Growth and Differentiation* (Symp. 25, Soc. Exp. Biol., Cambridge University, London, 1971).

³ Tettenborn, U., Dofuku, R., and Ohno, S., *Nature New Biology*, **234**, 37 (1971).

⁴ Gartler, S. M., Gandini, E., Hutchison, H. T., Campbell, B., and Zecchi, G., *Ann. Hum. Genet.*, **35**, 1 (1971).

⁵ Gardner, R. L., and Lyon, M. F., *Nature*, **231**, 385 (1971).

⁶ Deol, M. S., and Whitten, W. K., *Nature* (in the press).

⁷ Nesbitt, M. N., *Develop. Biol.*, **26**, 252 (1971).

tissues, other markers (such as isozymes) reveal a similar conglomerate of two cell types². Naturally occurring mosaics, such as those which arise according to the Lyon hypothesis through random *X*-inactivation in females, provide further examples^{3,4}. In every case, the chimaeric pattern records something of the developmental history of the individual. Indeed, it has been claimed that the assortment of cell types in the chimaeric differentiated tissue provides some crucial evidence about the mechanisms of differentiation and morphogenesis. In particular, Mintz¹ and Tettenborn *et al.*⁴ have sought to infer the initial numbers of specific tissue progenitors from observations of how often, in mosaic animals, those tissues are not mosaic, but homogeneous. We shall analyse this type of inference, and argue that the conclusions to which it properly leads do not have quite the significance that has been suggested.

We must first consider the various conceivable meanings of the phrase "the initial number of progenitors" of a specific tissue. Which generation of progenitor cells of the tissue does it refer to? Three broad possibilities immediately suggest themselves. (1) The very earliest generation of progenitors, that is, (a) trivially, in a non-chimaeric individual, the fertilized egg, or (b) in a chimaeric individual, the progenitors at the time when the chimaera was created. (2) The progenitors at the time when presumptive fate becomes predictable, that is, at the time when we can first be certain, on the basis of their location, which cells will either (a) contribute at least some progeny to the tissue in question (and perhaps other of their progeny to other tissues) or (b) contribute all of their progeny to the tissue in question. (Before this time, there may be random variation between different embryos of the given breed as to the fate of the progeny of equivalently placed cells, so that one cannot plot a fate map. One might say that (2a) corresponds to the earliest definite multi-valued fate map, and (2b) to the earliest definite single-valued fate map.) (3) The progenitors at some crucial early stage of their differentiation, for example at the time of determination or at the beginning of overt gross differentiation.

These three notions of "the initial number of progenitors" are interrelated. But we wish here to stress that the "initial number of progenitors" in sense (3) may be a much later and much larger group of cells than "the initial number of progenitors" in sense (1) or (2): the presumptive fate of a cell may be ordained by virtue of its position long before it differentiates. It will be suggested that experiments like those of Mintz¹ or of Tettenborn *et al.*⁴ give evidence about "initial numbers of progenitors" in senses (1b) and (2a) rather than (2b) or (3). They provide rather the same type of information as would a classical embryologist's vital staining experiment, in which he introduced the stain blindfold, in an uncertain quantity, and (in the parallel to the *X*-inactivation chimaera) at a time which he did not precisely know. The virtue of the chimaera is simply that its cells are marked by a label which does not fade, leak or get diluted. In special conditions this can make it uniquely valuable⁵.

The type of animal we have to consider is a mosaic of genetically different clones of cells. The clonal units, within which there is genetic concord, and between which there may be genetic discord, we shall call the "primary clones", for want of a better term; and we shall call the single progenitor of each such clone a "primary ancestor". In an *X*-inactivation mosaic, the primary ancestors are the cells of the generation in which heritable *X*-inactivation first occurred. In a double-embryo fusion chimaera, of the type created by Tarkowski⁶, Mintz¹ or McLaren and Bowman⁷, the primary ancestors are the two zygotes which give rise to the two components of the compound embryo. In the course of development, the cells of the primary clones may remain segregated, or they may mingle. The more they mingle, the more fine-grained the variegation in the adult, and the higher the probability of mosaicism in any given tissue or organ. Thus we must take cell mixing into account if, like Tettenborn *et al.*, or Mintz, we want to derive from the

Chimaeras and Cell Lineage in Development

CHIMAERAS (or "allophenic individuals") let us see the pedigree of cells. For instance, a blastocyst compounded from a black-coated-mouse embryo and a white-coated-mouse embryo may develop into a striped or piebald adult mouse¹. In other adult

measured frequency of mosaicism in some organ an estimate of the size of its primordium at an earlier stage of development.

Let us focus attention on some tissue T of a developed individual. Let A be the number of primary ancestors contributing at least some of their progeny to this tissue. The number A may vary from one individual to the next. We must therefore allow a range of different possible values for A , each associated with a probability of occurrence, p_A . Let f_A be the probability, for a given value of A , that all the A primary ancestors of the tissue T , and hence all its developed cells, are of the same specified type. Then for a randomly chosen individual, the probability P that the tissue T will be of the specified pure non-mosaic type is

$$P = \sum_A p_A f_A$$

where the sum over A ranges from 1 up to the total number N of primary ancestors in the embryo.

In a double-embryo fusion chimaera (for example Mintz's allopheic mouse), the experimenter usually chooses the two primary ancestors to be genetically different, so that the tissue T is guaranteed mosaic if it includes progeny of both, and non-mosaic, though of uncertain pure type, if it is derived from only one; hence

$$f_A = \begin{cases} \frac{1}{2} & \text{if } A=1 \\ 0 & \text{if } A=2 \end{cases}$$

and so

$$P = \frac{1}{2} p_1$$

In an X -inactivation mosaic, on the usual simple though questionable assumptions about random X -inactivation, there is a probability of $(1/2)^A$ that all the A primary ancestors of the tissue T inactivate the same specified X chromosome; hence

$$f_A = \left(\frac{1}{2}\right)^A$$

and so

$$P = \sum_{A=1}^N \left(\frac{1}{2}\right)^A p_A$$

The problematical factor is p_A , which depends on the amount of cell mixing and on the size and shape of the embryo and its parts. Rather than attempt the general case, we consider two extremes, which should make matters clear qualitatively.

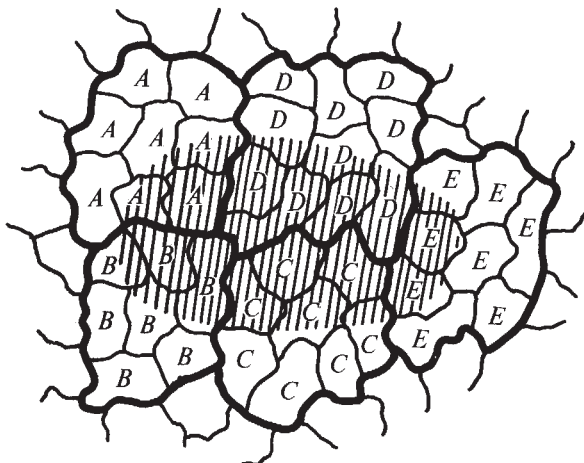


Fig. 1 The zone of cells fated to contribute at least some of their progeny to the tissue T is shaded. Each cell is marked with a letter to show which primary ancestor it is descended from. The boundaries of the primary clones are heavily drawn.

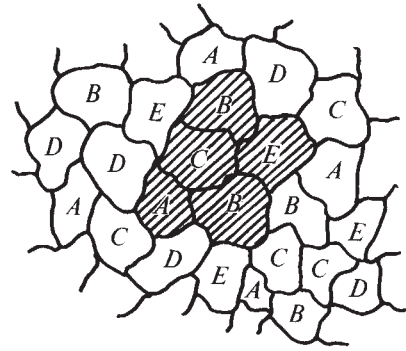


Fig. 2 The notation is the same as in Fig 1, except that there are no heavy boundaries to be marked between the primary clones.

First, suppose that there is no intermingling of cells in the generations that follow the primary ancestors. Then the initial mosaic pattern of primary ancestors begets a similar pattern of primary clones, each sharply demarcated from its neighbours. In Fig. 1, we symbolize a part of the animal at the earliest time t_c at which the presumptive fate of each cell is predictable to the extent that it is certain which cells will contribute at least some of their progeny to the tissue T ; that is, we show the initial set of progenitors of this tissue in sense (2a). It is clear that the number A of primary ancestors of the tissue T , and hence the probabilities p_A and P , are governed not by the absolute size of the primordium at the time t_c , but only by its size in relation to the sizes of the primary clones. In this case the frequency with which a given organ has a pure, non-mosaic phenotype gives an estimate of the number of primary ancestors which contribute to that organ, that is, it indicates the initial number of progenitors in sense (1b). But the initial set of progenitors in any other sense may be many times larger. In these circumstances, it would be rather an exaggeration to say that "(Tettenborn *et al.*'s) finding, and similar observations by Beatrice Mintz on the small number of progenitor cells giving rise to other organs and tissues, are of immense importance for theories about the determination of the fate of embryonic cells"⁸.

Second, suppose that there is a great deal of cell mixing in the early embryo, so that the primary clones are thoroughly intermingled at the time t_c . In Fig. 2 we again symbolize the tissue at that time. Let n be the number of cells at the time t_c which will contribute to the tissue T (that is, the initial number of progenitors of the tissue T , in the sense (2a)). These n cells are, by hypothesis, a random selection from the progeny of the N primary ancestors. One can work out the probability p_A that the n cells are altogether descended from just A of the primary ancestors. Assuming that all primary ancestors have the same number of progeny at time t_c , and that the n cells selected are only a small proportion of the entire population, we find

$$p_A = \frac{N!}{A!(N-A)!} \sum_{k=0}^A \frac{(-1)^{A-k}}{k!(A-k)!} \left(\frac{k}{N}\right)^n$$

This gives, for the double-embryo fusion chimaera (where $N=2$)

$$P = \frac{1}{2} p_1 = \left(\frac{1}{2}\right)^n$$

and for the X -inactivation chimaera (after some rearrangement)

$$P = \sum_{A=1}^N \left(\frac{1}{2}\right)^A p_A = \sum_{j=1}^N \left(\frac{1}{2}\right)^N \frac{N!}{j!(N-j)!} \left(\frac{j}{N}\right)^n$$

The mathematical details of the last expression are less important than its rough qualitative behaviour (which is in any case easy to divine without doing any calculations): if $n \gg N$, then very crudely

$$P \sim \left(\frac{1}{2}\right)^N$$

and if $n \ll N$, then very crudely

$$P \sim (\frac{1}{2})^n$$

In other words, P reflects the size of whichever set of cells is smaller; for the cells of the smaller set have the major chance of being all of one type. Thus if nothing is known about the comparative sizes of N and n , there is an ambiguity in the interpretation of P (for the X -inactivation chimaera, that is, but not for the double-embryo fusion chimaera).

The case of X -inactivation chimaeras with thorough cell mixing has been well analysed by Nesbitt⁹, though from a point of view rather different from ours; she shows how N and n can be worked out separately from the correlations between different tissues. It should, however, be emphasized that n , found either in this way, or by using observations of P together with some independent estimate of N , is only the number of progenitors of the tissue at the time t_c when their presumptive fates become fixed; that is, it is only the initial number of progenitors in the sense (2a). Presumptive fate maps are, of course, important, and we do not wish to decry the value of mosaics for their investigation. *Drosophila gynandromorphs* in particular have been elegantly exploited to plot fate maps on the blastoderm of the developing fly⁵.

The above discussion leads us to ask how far cells really intermingle in the early embryo. In general it seems that they do so rather little. For example, Rosenquist's¹⁰ maps of the origin and movement of nephrogenic cells in the chick embryo suggest that there is not much random diffusion: cells which start in a cluster stay in a cluster, even though its shape and position may change. The first of the two extreme cases we have discussed is therefore probably closer to reality than the second. Nesbitt and Gartler³, in their review, lend some support to this opinion.

Consider now the experiments of Tettenborn *et al.*⁴, who have studied the kidney proximal tubule cells of female mice heterozygous for the X -linked *Tfm* mutation. According to the Lyon hypothesis, we should expect that at some stage of development X -inactivation takes place; each cell then present adopts either the *Tfm* or the normal phenotype, which it passes on to its clone of descendants. Tettenborn *et al.* find that in roughly one case in 2^4 or 2^5 , all the kidney proximal tubule cells in the adult mouse are of the *Tfm* type. Hence they infer that these cells "are the descendants of only four or five embryonic progenitor cells"; and they imply, by the first paragraph of their article, that they are referring to the number of progenitors at the time of what they call "commitment". According to our analysis, however, provided that there is indeed not much cell mixing, the experiments of Tettenborn *et al.* reveal only that four or five of the cells present at the time of X -inactivation contribute at least some of their progeny to the kidney proximal tubules (compare our case (1b)). Very little can be learnt about the number of progenitors of these tissues during the crucial biochemical steps of determination as kidney; and this holds true however much the embryonic cells may mix.

Our analysis has a bearing also on some analogous deductions by Mintz¹ to do with the clonal origin of the embryo. Mintz observes that, of the adult mice that develop from compound allophenic blastocysts, one in four is not a mosaic individual, but has a pure phenotype. She tentatively infers that when the embryo proper first becomes differentiated from the trophoblast, it consists of just three randomly assorted cells. Our chief objection here is that Mintz's inference depends on an implicit and unsubstantiated assumption that the two allophenic cell types were thoroughly intermingled at the time of the first "differential genetic activities" of the embryo proper. If the degree of mixing is slight, the observed frequency of non-mosaic individuals tells us almost nothing about the number of initiator cells of the embryo proper at that time.

Mintz furthermore seems to assert that the number of initiator cells of the embryo proper, and indeed of any tissue,

is "genetically determined" and invariant between individuals of similar genotype. (She writes that from her studies "it is apparent that a specific clonal numerology exists for each cell type"¹.) Even if we grant the assumption of thorough cell mingling, this statement is unwarranted. While the relative frequencies of mosaic and non-mosaic individuals or tissues may indicate the average number of initiator cells, it gives no clue to the natural range of variation of that number. The 1:3 proportion of non-allophenic to allophenic mice may for instance arise because there are sometimes two, sometimes three, and sometimes four initiator cells, with probabilities in the ratio 1/4:1/4:1/2.

Finally, we would question a general claim that Mintz makes about the developmental significance of mosaic patterns. She writes¹: "The most revealing markers are those that allow cells of the respective genotypes to be visualized *in situ*, within tissues that are relatively fixed in space. Contiguous cells that form a unit 'patch' of one or the other alternative allelic phenotype are likely to have descended clonally from a single genetically determined precursor cell. The geometry of the clonal area reflects the progressive deployments of the cells that comprise it. Thus antecedent genetic and developmental events can be reconstructed from mosaic patterns in allophenic adults". Here again there is confusion as to which generation of precursors is being referred to. In the specified conditions, that is, when cells are fairly immobile, the pattern of patches in the adult reflects only the pattern of single cells at the time when cell mixing effectively stopped. In general this time is quite different from the time of the key biochemical events responsible for differentiation (Mintz's "genetic determination"); and correspondingly the number and arrangement of tissue precursor cells is quite different. There may of course be some special tissues in which the end of cell mixing is associated with the beginning of differentiation; melanoblasts, with their characteristic migratory phase, might perhaps be a case in point. For them biochemical determination may possibly be roughly coincident with the phase of migration from the neural crest, followed by colonization of the flanks¹¹. If the melanoblasts migrate briskly far and wide, and so settle after a short while in a pattern which is random with respect to their initial arrangement in the neural crest, then the adult coat may indeed record the distribution of individual melanoblasts soon after the time of their determination. The premise is, however, doubtful. McLaren¹² has suggested that there could be a tendency for like cells to aggregate in the neural crest. Then the patches of coat colour might correspond to large families of primordial melanoblasts which stayed together in their journey from a multicellular patch in the neural crest. It may be possible to discriminate between the models based on random assortment and on selective aggregation using the analysis of Wolpert and Gingell¹³.

In conclusion, experiments like those of Tettenborn *et al.* or of Mintz reveal only the relative size of the group of cells giving rise to a tissue, compared with the average mosaic patch size, at the time when the presumptive fate of those progenitors first became definite. From this type of investigation the number of progenitors at the time of determination, that is, at the time of the first, decisive step of differentiation, cannot be deduced. The interpretation of the experiments hangs on the role of cell mixing. In another paper¹⁴ we shall try to throw some light on this important process by calculating how the descendants of a given cell become distributed within a homogeneous, growing, mobile population.

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J. H. LEWIS
D. SUMMERBELL
L. WOLFERT

*Department of Biology as Applied to Medicine,
Middlesex Hospital Medical School,
London W1P 6DB*

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- ¹ Mintz, B., in *Control Mechanisms of Growth and Differentiation* (Symp. 25, Soc. Exp. Biol., Cambridge University, London, 1971).
- ² Moore, W. J., and Mintz, B., *Develop. Biol.*, **27**, 55 (1972).
- ³ Nesbitt, M. N., and Gartler, S. M., *Ann. Rev. Genet.*, **5**, 143 (1971).
- ⁴ Tettenborn, U., Dofuku, R., and Ohno, S., *Nature New Biology*, **234**, 37 (1971).
- ⁵ Garcia-Bellido, A., and Merriam, J. R., *J. Exp. Zool.*, **170**, 61 (1969).
- ⁶ Tarkowski, A. K., *Nature*, **190**, 857 (1961).
- ⁷ McLaren, A., and Bowman, P., *Nature*, **224**, 238 (1969).
- ⁸ *Nature New Biology*, **235**, 33 (1972).
- ⁹ Nesbitt, M. N., *Develop. Biol.*, **26**, 252 (1971).
- ¹⁰ Rosenquist, G. C., *J. Embryol. Exp. Morphol.*, **24**, 367 (1970).
- ¹¹ Rawles, M. E., *Physiol. Rev.*, **28**, 383 (1948).
- ¹² McLaren, A., in *Handbook of Molecular Cytology* (edit. by Lima-de-Faria, A.) (North-Holland, Amsterdam, 1969).
- ¹³ Wolpert, L., and Gingell, D., *J. Theoret. Biol.*, **29**, 147 (1970).
- ¹⁴ Lewis, J. H., *J. Theoret. Biol.* (in the press).

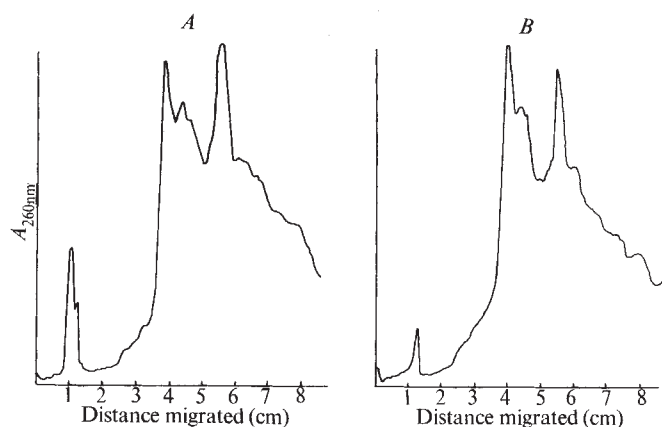


Fig. 2 The effect of DNAase treatment. Scans of 2.6% gels loaded with 200 μ g of total nucleic acid from the killer strain KB. A, Scan of the sample incubated with heat killed DNAase; B, DNAase treated sample.

A New Species of Double-stranded RNA from Yeast

A NEW species of double-stranded RNA has been detected in certain strains of bakers' yeast, *Saccharomyces cerevisiae*, during investigations of the chemical nature of the cytoplasmic genetic determinants concerned with the killer character in yeast. There are three phenotypes with respect to this killer phenomenon: killer, sensitive and neutral. Killer cells kill sensitive cells by secreting into the medium a toxic polypeptide^{1,2} to which they themselves are immune. Neutral cells neither kill nor are killed. The phenomenon is under the control of at least one nuclear gene and two types of cytoplasmic determinant, (*k*) and (*n*), which confer the killer and neutral phenotypes respectively³⁻⁵.

In attempts to detect a species of nucleic acid present in killer, neutral and sensitive cells which might correspond to these cytoplasmic determinants, we compared DNA preparations from all three phenotypes with negative results⁶. Total nucleic acid extracts were then prepared. Cells were grown for three days on yeast complete medium⁷ (30 g wet weight), and disrupted in a Hughes press. Total nucleic acid was extracted essentially by the phenol method as described by Kirby and Cook⁸. Ethanol was added and the final precipitate was dissolved in 0.1 M acetate buffer, pH 6.0, and centrifuged at

100,000g for 2 h at 4° C to remove high molecular weight polysaccharide. The final solution had a ratio of absorption at 260 : 230 : 280 nm of 1.0 : 0.395 : 0.445 respectively and contained up to 300 mg RNA and up to 10 mg DNA as assayed by the orcinol⁹ and diphenylamine¹⁰ methods. Preparations were then examined by acrylamide gel electrophoresis, according to the method of Loening¹¹. From 5–200 μ g of the sample were added in from 5–100 μ l. total solution to 2.6% gels. Electrophoresis was carried out at 7 V/cm and 5 mA/gel for 3–4.5 h. Gels were scanned using a 'Joyce Loebel Chromoscan' and stained in 0.01% Toluidine Blue for 12 h.

Scans were carried out on gels loaded with total nucleic acid taken from a sensitive strain (76a) and a killer strain (KB). The killer strain showed a sharp peak with a slightly higher mobility than the DNA (see Fig. 1, peak, X). The percentage of the peak X fraction in the total preparation was estimated at 0.5%. The gel profile of a preparation from a neutral strain (N2) resembled that of the killer strain.

Enzyme treatments were then carried out to determine the nature of the peak X fraction. 25 μ g DNAase (Sigma, electrophoretically pure) in 0.1 M morpholino-sulphonic acid buffer, pH 6.9, containing 3 mM magnesium acetate were added to 1 ml. of total nucleic acid extract from killer cells, the mixture incubated for 30 min at 0° C, and 200 μ g aliquots examined by acrylamide gel electrophoresis. In the control, the gel profile was unaltered, but in the DNAase treated sample the DNA peak was absent, leaving the peak X fraction and the r-RNA intact (Fig. 2).

Pancreatic RNAase was heated at 85° C for 10 min to inactivate any DNAase present. 50 μ g RNAase were added to 1 ml. of total nucleic acid extracted from killer cells, the mixture incubated at 0° C for 30 min, and 200 μ g aliquots applied to acrylamide gels. Enzyme treatment removed both the X peak and the r-RNA, leaving the DNA intact (Fig. 3).

The X peak fraction is therefore an RNA species. Extracts were then treated with a range of concentrations of RNAase to ascertain whether the molecule was single or double-stranded. 1 ml. aliquots of total nucleic acid extract from killer (KB) cells in 0.15 M NaCl–0.015 M sodium citrate (SSC), pH 7.0, were incubated with from 0.1 to 50 μ g/ml. of heat treated pancreatic RNAase for 30 min at 0° C. Samples of 200 μ g were loaded on to acrylamide gels and electrophoresis carried out for 3.5 h. The results are shown in Table 1.

Peak X, unlike r-RNA, is resistant to high concentrations of RNAase, a property characteristic of double-stranded RNA¹². Three other properties of peak X suggest double-strandedness. First, it has a very similar mobility to DNA on polyacrylamide gels, double-stranded molecules tending to behave differently from single-stranded molecules under similar conditions¹³. Second peak X and DNA both stain pink with

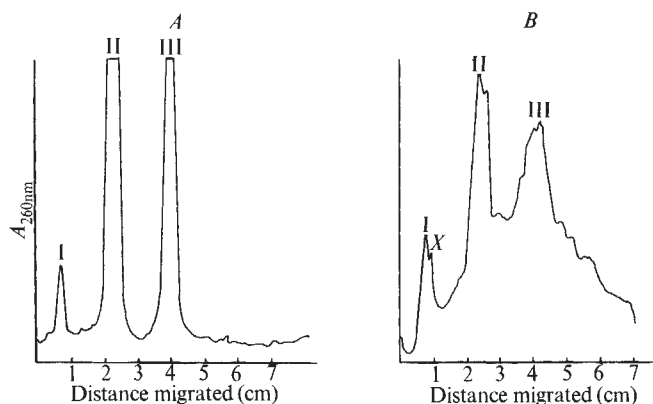


Fig. 1 Nucleic acid from strains KB and 76a. Scans of 2.6% gels loaded with 100 μ g of total nucleic acid from 76a sensitive strain (A) and KB killer strain (B). I, II and III represent DNA, large r-RNA sub-unit and small r-RNA sub-unit respectively. In addition to peak X the two preparations differ in the amount of breakdown undergone by r-RNA. This breakdown was found in variable amounts in all preparations from killer cells. Its significance is unknown.

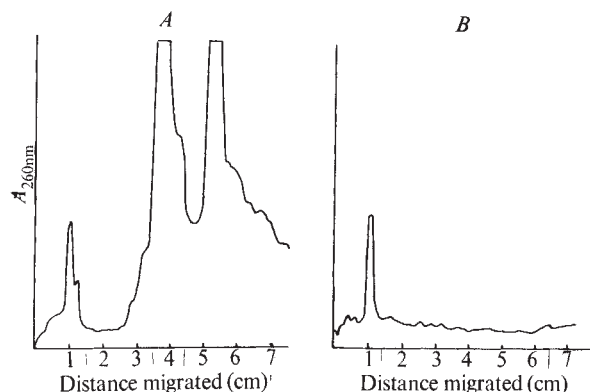


Fig. 3 The effect of RNAase treatment. Scans of 2.6% gels loaded with 200 μ g total nucleic acid from the killer strain KB. A, Sample incubated with saline; B, RNAase treated sample (1 μ g/ml.).

Toluidine Blue, whereas r-RNA stains purple; pink staining is characteristic of double-stranded molecules. Third, purified X-RNA induces the formation of interferon in mouse L-cells (A. H. Herring and K. E. Mogensen, personal communication); also a characteristic of double-stranded molecules.

Table 1 Effect of RNAase Concentration on r-RNA and Peak X

Conc. RNAase μ g/ml.	Peak X	r-RNA
50.0	—	—
10.0	Half-removed	—
1.0	+	—
0.5	+	—
0.1	+	A trace

+, Present. —, Not present.

No virus-like particles have been detected in yeast strains containing peak X, and it is assumed that the yeast system is not analogous to the virus-like particles containing double-stranded RNA in *Penicillium* and *Aspergillus* spp.^{14–17}. Lack of a protein coat does not preclude a viral-like origin for X-RNA. Potato spindle tuber virus¹⁸ and citrus exocortis virus¹⁹ have many of the properties expected for naked double-stranded RNA molecules.

X-RNA cannot yet be directly or indirectly identified with the killer and neutral cytoplasmic determinants. Although all killer and neutral strains possess it, it is lacking in some sensitive strains. Sensitive strains in which it has been found have been derived by mutation from killer strains, so it is conceivable that killing ability has been lost without concomitant loss of the X-RNA.

E. A. BERRY

E. A. BEVAN

Department of Plant Biology and Microbiology,
Queen Mary College,
University of London,
Mile End Road,
London E1 4NS

Received April 26, 1972.

- ¹ Woods, D. R., and Bevan, E. A., *J. Gen. Microbiol.*, **51**, 115 (1968).
- ² Bussey, H., *Nature New Biology*, **235**, 73 (1972).
- ³ Bevan, E. A., and Somers, J. M., *Genet. Res., Camb.*, **14**, 71 (1969).
- ⁴ Somers, J. M., and Bevan, E. A., *Genet. Res., Camb.*, **13**, 71 (1969).
- ⁵ Bevan, E. A., Somers, J. M., and Theivendirarajah, K., *Proc. Eleventh Intern. Bot. Cong., Seattle* (1969).
- ⁶ Berry, E. A., thesis, London University (1971).
- ⁷ Cox, B. S., and Bevan, E. A., *New Phytol.*, **61**, 342 (1962).
- ⁸ Kirby, K. S., and Cook, E. A., *Biochem. J.*, **104**, 254 (1967).
- ⁹ Ogur, M., and Rosen, G., *Arch. Biochem.*, **25**, 262 (1950).
- ¹⁰ Burton, K., *Biochem. J.*, **62**, 315 (1956).
- ¹¹ Loening, U. E., *Biochem. J.*, **102**, 251 (1967).
- ¹² Ralph, R. K., *Adv. Virus Research*, **15**, 61 (1969).

- ¹³ Fisher, M., Patricia, and Dingman, C. Wisley, *Biochem.*, **10**, 1895 (1971).
- ¹⁴ Ellis, L. F., and Kleinschmidt, W. J., *Nature*, **215**, 649 (1967).
- ¹⁵ Banks, G. T., Buck, K. W., Chain, E. B., Darbyshire, J. E., and Himmelweit, F., *Nature*, **222**, 89 (1969).
- ¹⁶ Banks, G. T., Buck, K. W., Chain, E. B., Darbyshire, J. E., Himmelweit, F., Ratti, G., Sharpe, T. J., and Planterose, D. N., *Nature New Biology*, **227**, 505 (1970).
- ¹⁷ Lhoas Pol, *Nature New Biology*, **236**, 86 (1972).
- ¹⁸ Diener, T. O., and Raymer, W. B., *Science*, **158**, 378 (1967).
- ¹⁹ Semanik, J. S., and Weathers, L. G., *Virology*, **36**, 326 (1968).

Cyclic AMP Released by Rat Testis during Gonadotrophin Stimulation *in vitro*

THE stimulation of testosterone secretion from testis interstitial cells by luteinizing hormone (LH) and human chorionic gonadotrophin (hCG) is believed to depend on cyclic AMP formation, as is the case with other steroidogenic tissues¹. Addition of cyclic AMP to slices of rat² and rabbit testis³ stimulates testosterone production, and LH increases adenylate cyclase activity in the testis of dog⁴ and rat⁵. We have observed that the isolated rat testis is extremely sensitive to LH and hCG stimulation *in vitro*, responding to low levels of hCG (1 ng/ml.) with maximum release of testosterone into the incubation medium⁶. In contrast, the teased rat testis responds poorly to gonadotrophic stimulation, and adenylate cyclase in testis homogenates is relatively insensitive to large doses of LH or hCG⁷.

To define more clearly the role of cyclic AMP in gonadotrophin stimulation of androgen production by the testis, we have examined testosterone release in response to cyclic AMP and its dibutyl derivative *in vitro*, and the formation of cyclic AMP by the testis in response to stimulation with chorionic gonadotrophin *in vitro*. These studies were performed during incubation of intact decapsulated adult rat testes in Krebs–Ringer–bicarbonate glucose buffer at 34° C under 95% O₂:5% CO₂. Testosterone production during incubation was measured by direct radioimmunoassay of aliquots of diluted incubation media⁶; cyclic AMP formation was measured in the presence of 5 mM theophylline, by conversion from ¹⁴C-adenine^{7,8} and by radioimmunoassay of cyclic AMP⁹ in incubation media and acetic acid extracts of tissue.

Addition of increasing doses of cyclic AMP or its dibutyl derivative to isolated rat testes caused a graded increase in testosterone production (Fig. 1). Dibutyl cyclic AMP was about fifty times more effective than cyclic AMP in promoting testosterone formation; this potency ratio is similar to that observed in certain other tissues, such as liver¹⁰. In each case the response to the nucleotide occurred over approximately a

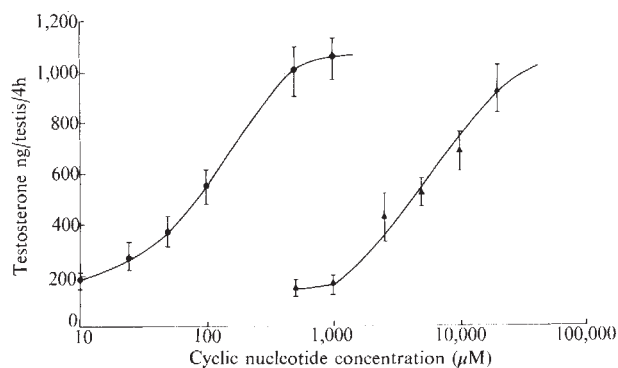


Fig. 1 Testosterone synthesis by the isolated rat testis in the presence of increasing doses of cyclic AMP (Δ) and dibutyl cyclic AMP ($\bullet$). Each point represents the mean $\pm$ s.e. of quadruplicate determinations.

ten-fold concentration range, as did the previously observed testosterone response to LH and hCG *in vitro*⁶.

During incubation of isolated rat testes with hCG, progressive release of substantial quantities of cyclic AMP into the incubation medium was observed, both by the ¹⁴C-adenine incorporation method and by radioimmunoassay of total cyclic AMP (Fig. 2). In each case, the release of cyclic AMP from the unstimulated testis was minimal or undetectable, while addition of hCG caused rapid release of the nucleotide into incubation media. At the same time, tissue levels of cyclic AMP remained relatively constant or rose irregularly (Fig. 3). In all experiments the extent of cyclic AMP release was greater than the concurrent change detected in tissue cyclic AMP content, with progressive elevation of medium cyclic AMP content from 5 to 30 min after addition of hCG. Release of cyclic AMP during hCG stimulation preceded the earliest observed rise of testosterone formation *in vitro* at 10–15 min⁶, consistent with an intermediate role of the nucleotide in gonadotrophin-induced androgen synthesis by the Leydig cell.

The parallel changes in ¹⁴C-adenine incorporation and total cyclic AMP release measured by radioimmunoassay indicate that newly synthesized cyclic AMP comprised a constant proportion of the nucleotide released in response to hCG over the first 30 min of stimulation. The marked and rapid release of cyclic AMP from the hCG-stimulated testis in the presence of more modest intracellular changes is analogous to the release of cyclic AMP into liver perfusate in the presence of glucagon and epinephrine concentrations that do not elevate tissue cyclic AMP¹¹. Hormonally-induced acute cyclic AMP changes in fat cells, however, are not accompanied by corresponding rises in medium concentrations of the nucleotide¹². In some reported studies, incubated tissue and media have not been separated before extraction of cyclic AMP for assay. It is also possible that medium concentrations of cyclic AMP may have contributed to the results observed during hormonal stimulation. For studies on the response of interstitial cells to gonadotrophins, release of cyclic AMP into incubation medium from the isolated intact rat testis provides

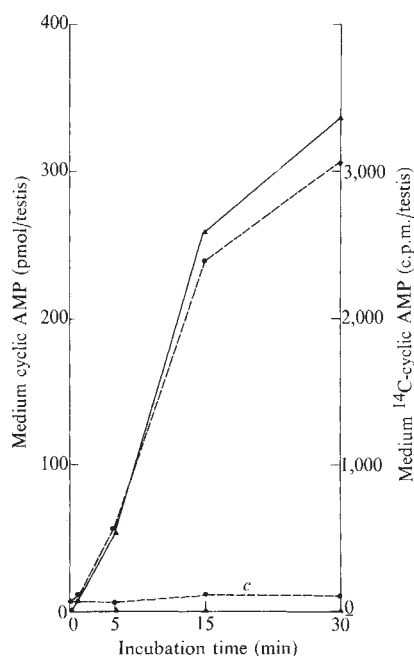


Fig. 2 Release of cyclic AMP into the incubation medium during *in vitro* stimulation of rat testes with hCG at 500 ng/ml. Cyclic AMP formation from endogenous ¹⁴C-ATP and the total release of immunoreactive nucleotide gave similar patterns of release during incubation. Control concentrations of cyclic AMP measured by radioimmunoassay were undetectable during the first 30 min of incubation. Each point represents the mean of triplicate determinations. ▲—▲, pmol/testis; ●—●, c.p.m./testis; c, control.

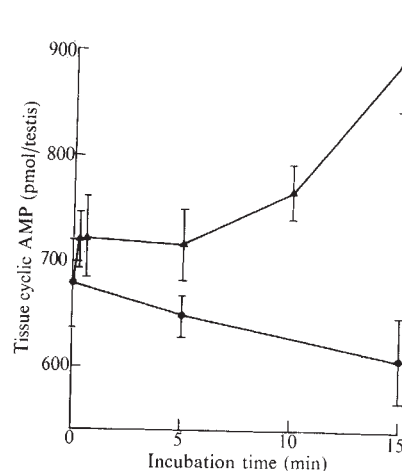


Fig. 3 Tissue concentrations of cyclic AMP during incubation of rat testes with hCG at 500 nm/ml. Each point represents the mean $\pm$ s.e. of triplicate determinations. ▲, hCG; ●, control.

a rapid and sensitive index of hormonal activation *in vitro*. More recently, detectable responses in cyclic AMP release have been observed in testes exposed to concentrations of hCG as low as 1 ng/ml, which is well within the physiological range of hCG and LH concentration in body fluids. The results of these studies are consistent with the view that activation of adenylate cyclase is an important early step in the action of LH and hCG on testosterone synthesis by the Leydig cell.

K. J. CATT
K. WATANABE
M. L. DUFAY

Reproduction Research Branch,
National Institute of Child Health and Human
Development,
National Institutes of Health,
Bethesda,
Maryland 20014

Received May 3, 1972.

- ¹ Robinson, G. A., Butcher, R. W., and Sutherland, E. W., *Ann. Rev. Biochem.*, **37**, 149 (1968).
- ² Sandler, R., and Hall, P. F., *Endocrinology*, **79**, 647 (1966).
- ³ Eik-Nes, K. B., *Recent Prog. Hormone Res.*, **25**, 517 (1971).
- ⁴ Murad, F., Strauch, B. S., and Vaughan, M., *Biochim. Biophys. Acta*, **177**, 591 (1969).
- ⁵ Kuehl, F. A., Panatelli, D. J., Tarnoff, J., and Humes, J. L., *Biology of Reproduction*, **2**, 154 (1970).
- ⁶ Dufay, M. L., Catt, K. J., and Tsuruhara, T., *Endocrinology*, **90**, 178 (1972).
- ⁷ Dufay, M. L., Catt, K. J., and Tsuruhara, T., *Biochim. Biophys. Acta*, **252**, 574 (1971).
- ⁸ Kuo, J. F., and De Renzo, E. C., *J. Biol. Chem.*, **244**, 2252 (1969).
- ⁹ Steiner, A. L., Kipnis, D. M., Utiger, R., and Parker, C., *Proc. US Nat. Acad. Sci.*, **64**, 367 (1969).
- ¹⁰ Henion, W. F., Sutherland, E. W., and Pasternak, T., *Biochim. Biophys. Acta*, **148**, 106 (1967).
- ¹¹ Broadus, A. E., Hardman, J. G., Kaminsky, N. I., Ball, J. H., Sutherland, E. W., and Liddle, G. W., *Ann. New York Acad. Sci.*, **185**, 50 (1971).
- ¹² Murad, F., Manganiello, V., and Vaughan, M., *J. Biol. Chem.*, **245**, 3352 (1969).

Abnormal Concentrations of Dopamine in a *Drosophila* Mutant

THE body colour mutant *tan* of *Drosophila melanogaster* has been shown to possess a defect in its visual system, as evidenced by an abnormal electroretinogram (ERG)^{1,2}. As part of a search for a chemical correlate of this mutation, the concentration of dopamine in *tan-1* flies was compared with that in flies of normal body colour. Dopamine is an important intermediate in cate-

cholamine biosynthesis³ as well as in the tanning pathway of at least some insects⁴.

The gene for white eyes was used in combination with normal and mutant body colour genes to minimize contaminating fluorescence produced by eye screening pigments. (The *white* gene does not produce any abnormality in the ERG.) Flies were reared on cornmeal medium at 25° C. Dopamine was extracted by homogenizing a sample of twenty-five or thirty flies, previously frozen in liquid nitrogen, in a glass homogenizer containing 0.3 ml. of 0.5 N perchloric acid at room temperature. The homogenate was transferred to a clean tube and the homogenizer rinsed successively with 0.1 ml. and 0.05 ml. of 0.5 N perchloric acid. The rinses were combined with the homogenate and the solution centrifuged in the cold at 10,000 r.p.m. for 15 min in a Sorvall SS-34 or HB-4 rotor. The supernatant was removed and to it were added 50 μ l. of 1 M sodium phosphate buffer, pH 7, and enough 2 N potassium carbonate to bring the pH of the solution to 6. The solution was centrifuged as above in the cold; the clear supernatant was transferred to a tube containing 60 μ l. of 'Amberlite CG-50' resin prepared according to the method of Eccleston *et al.*⁵ and washed with 1.5 ml. of glass distilled water before use. The dopamine was adsorbed to the resin by shaking the tube gently on a Vortex mixer for 1 min. The resin was allowed to settle and the supernatant removed; the resin was washed four times with 1.5 ml. of glass distilled water and the dopamine eluted by shaking the resin with 0.4 ml. of 2.2 N perchloric acid on a Vortex mixer as before. The resin was again allowed to settle and the supernatant transferred to a clean tube; to it were added 50 μ l. of 1 M sodium phosphate, pH 7, and enough 2 N potassium carbonate to bring the pH to 6.5. The solution was centrifuged in the cold as above to remove the perchlorate precipitate; the supernatant was removed, mixed thoroughly, and divided in half for the assay, one half serving as sample and the other as a faded blank. The oxidation of dopamine to its fluorophore was carried out according to the method of Carlsson and Waldeck⁶, using 10 μ l. of iodine solution and 50 μ l. of alkaline sulphite and 6 N acetic acid for each sample tube (the volumes of reagents used for the faded blanks were adjusted accordingly). After completion of the oxidation procedure, the tubes were incubated at 72° C. for 45 min to increase the fluorescence and then allowed to cool. Fluorescence was determined in a Farrand spectrofluorometer at 330 nm (excitation) and 390 nm (emission), uncorrected. The assay was linear to at least 380 fluorescence units; using both internal and external standards, one fluorescence unit was found to be equivalent to approximately 1 ng of dopamine.

Table 1 (top) gives the concentrations of dopamine in 0–1 day old flies with normal body colour and with the *tan* mutation. The concentration of dopamine in *tan-1* flies is about 0.6 that in normal flies.

Table 1 Concentrations of Dopamine in Adult Flies and Late Pupae

Genotype	Concentration (in arbitrary fluorescence units per animal $\pm$ s.d.)	No. of determinations
Adult flies 0–1 days after emergence		
white	4.6 $\pm$ 0.6	6
white, tan	2.7 $\pm$ 0.4	5
Late pupae		
white	10.0 $\pm$ 0.7	5
white, tan	7.2 $\pm$ 0.6	3

Twelve or fifteen animals were used for the sample and faded blank for each determination. One fluorescence unit is equivalent to approximately 1 ng of dopamine.

To investigate whether this defect persists throughout development, dopamine concentrations were measured at various stages. In late third-instar larvae, 0–1 day old pupae, and 2–3 day old pupae, no detectable dopamine was found (less than 0.2 fluorescence unit per animal). In very late pupae (with some eye and wing coloration visible through the puparium), detectable

amounts of dopamine were present. The results of several determinations are given in Table 1 (bottom). At this stage, as in adult flies, the concentration of dopamine in *tan* is abnormally low.

To determine whether a low concentration of dopamine is routinely correlated with light body colour, adults of other strains exhibiting light body colour mutations (*yellow*⁷ and *spook*) but having normal electroretinograms were tested. (The mutant *spook* is a very pale body colour mutant on the X chromosome isolated by Mr Gary Scheidt.) The dopamine concentrations of these strains were within $\pm 8\%$ of normal. Another allele of *tan* (*tan-5*, which has an ERG defect similar to that of *tan-1*) had 58% of the normal amount of dopamine. Thus, the dopamine abnormality in *tan* shows a specific correlation with the electrical defect in its visual system.

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RONALD J. KONOPKA*

Division of Biology,
California Institute of Technology,
Pasadena,
California 91109

Received June 5, 1972.

*Present address: Department of Biological Sciences, Stanford University, Stanford, California 94305.

¹ Hotta, Y., and Benzer, S., *Nature*, **222**, 354 (1969).

² Pak, W. L., Grossfield, J., and White, N., *Nature*, **222**, 351 (1969).

³ Seiler, N., Demisch, L., and Schneider, H., *Angew. Chem., Intern. Edit.*, **10**, 51 (1971).

⁴ Karlson, P., and Sekeris, C., *Nature*, **195**, 183 (1962).

⁵ Eccleston, D., Ashcroft, G., Crawford, T. B., and Loose, R., *J. Neurochem.*, **13**, 93 (1966).

⁶ Carlsson, A., and Waldeck, B., *Acta Physiol. Scand.*, **44**, 293 (1958).

⁷ Lindsley, D., and Grell, E. H., *Genetic Variations of Drosophila melanogaster* (Carnegie Institution, Washington, 1967).

Neonatal Androgenization and Aggression in the Male Golden Hamster

ADULT male mice and rats do not readily show female sexual behaviour following castration and treatment with oestrogen and progesterone. This seems to be the result of androgens secreted during the postulated "critical period" of brain development, acting on neural structures responsible for the expression of sex-typical behaviour^{1,2}. The golden hamster is anomalous since castrated adult males readily show lordosis in response to oestrogen/progesterone priming^{3,4}. This response is prevented, however, if exogenous androgens are administered immediately after birth^{5,6}.

The hamster also differs from other laboratory rodents in its aggressive behaviour. In most laboratory rodents the males are more aggressive than females^{7,8}, but both sexes of the golden hamster fight, and females will readily attack and defeat males. Furthermore, castrated males exhibit increased aggression in response not only to the administration of androgen but also to ovarian implantation⁹ or the administration of ovarian steroids^{10,11}. Aggression towards females can be activated in the normal male only by heterotypical sex hormones^{9,10}.

The social behaviour of the golden hamster¹² and the procedures used for this experiment¹³ have been described in detail elsewhere. Infant hamsters were injected subcutaneously on the day of birth with either 300 μ g testosterone propionate (TP) in 0.05 ml. arachis oil, or the oil vehicle alone. All members of the litter received the same treatment. When 100–140 days old, 13 TP-treated males and 15 oil-treated males were matched with untreated dioestrous females of similar body weight. Both animals were taken from their home cages

and placed in a third cage for a ten minute observation period on three successive days.

The golden hamster's aggressive behaviour includes not only overt components such as attacking, biting and chasing, but also ritualized postures of offence. No qualitative distinction has been made between these components and the frequency of aggressive behaviour per test has been represented as the number of times any aggressive element was exhibited during the ten minute observation period.

Control males showed the expected pattern of behaviour, that is, significantly less aggression than the intact females with which they interacted (Table 1). The males given TP showed higher, albeit not significantly different, levels of aggression than their female partners. The difference in the frequency of aggression shown by the two groups of males was significant ($t=3.94$, $P<0.01$).

After these tests, all males were castrated and retested while receiving (i) no treatment, (ii) 1,000 μg TP per day. The pairs of animals were again observed for ten minutes on each of three consecutive days (i) at least two weeks after castration and (ii) during the last three of ten days of treatment with TP. Each experimental animal was allocated a new female partner (matched for body weight) for each set of tests.

Table 1 Average Frequency per Test of Aggressive Behaviour shown by Male Golden Hamsters and their Female Partners

Sex	Treatment at birth	Sample number	Frequency of aggressive behaviour		
			No treatment	Castrated	Castrated + TP
Males	Oil injection	15	0.38	0.47	0.62
Females	Nil		1.33	1.42	1.60
<i>P</i>			*	†	*
Males	TP injection	13	1.59	0.59	1.07
Females	Nil		1.15	3.23	0.86
<i>P</i>			N.s.	†	N.s.

* $P<0.05$.

† $P<0.01$.

Student's paired *t* test.

The males were injected at birth with oil or with 300 μg testosterone propionate (TP). They were tested when receiving no treatment, castrated with no treatment, or castrated and receiving 1,000 μg testosterone propionate per day.

These tests (Table 1) show that castration and subsequent treatment with TP had no effect on the aggressive behaviour of the control males or their female partners, thus confirming previous findings on normal males¹⁰. Castration, however, significantly decreased the frequency of aggression shown by the neonatally androgenized males ($t=2.65$, $P<0.02$), while the females showed significantly more frequent aggression ($t=2.37$, $P<0.05$). Administration of androgen to the males resulted in both sexes re-attaining levels of aggression comparable with those observed prior to castration. Although the increase in aggression of the neonatally androgenized males in response to TP was not significant, this group showed significantly more aggression than the control males given the same treatment ($t=2.26$, $P<0.05$).

Administration of exogenous androgen to newborn male hamsters seems to increase their sensitivity to androgens as adults and results in the exhibition of behavioural characteristics comparable with normal, untreated males of other rodent species. They no longer show less aggression than females, and respond to changing levels of circulating androgens resulting from castration and treatment with TP. This complements previous findings that, whereas the adult castrate male golden hamster can display either male or female sexual behaviour depending on the hormones administered to it^{3,4}, the ability to display lordosis is suppressed in neonatally androgenized males^{5,6}. It seems, therefore, that the male golden

hamster has a system of neural differentiation comparable with the male rat or mouse, in which the presence of androgens during development facilitates the display of male behaviour in adulthood and suppresses the display of female behaviour. In the normal male hamster, however, such "masculinization" of the central nervous system seems to be incomplete. The secretion of androgens by the golden hamster may be less than in the rat or mouse, or the threshold for programming neural structures may be higher in this species. Nothing is known of the production of androgens in the golden hamster, but if the hypothesis is correct, differences between steroid production and uptake and utilization in the central nervous system of the golden hamster and other laboratory rodents in foetal and neonatal life could provide some understanding of sex-typical neural differentiation.

A. P. PAYNE

HEIDI H. SWANSON

Department of Anatomy,
The Medical School,
Birmingham B15 2TJ

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- ¹ Barraclough, C. A., *Endocrinology*, **68**, 62 (1961).
- ² Harris, G. W., *Endocrinology*, **75**, 627 (1964).
- ³ Swanson, H. H., *J. Reprod. Fert.*, **21**, 183 (1970).
- ⁴ Tiefer, L., and Johnson, W. A., *J. Endocr.*, **51**, 615 (1971).
- ⁵ Swanson, H. H., in *Influences of Hormones on the Nervous System*, *Proc. Int. Soc. Psychoneuroendocrinology*, Brooklyn 1970, 424 (Karger, Basel, 1971).
- ⁶ Swanson, H. H., and Crossley, D. A., in *Hormones in Development* (edit. by Hamburg, M., and Barrington, E. J. W.), 677 (Appleton-Century-Crofts, New York, 1971).
- ⁷ Beach, F. A., *Hormones and Behaviour* (Hoeber, New York, 1948).
- ⁸ Guhl, A. M., in *Sex and Internal Secretions* (edit. by Young, W. C.), 2, 1240 (Baillière, Tindall and Cox, London, 1961).
- ⁹ Payne, A. P., and Swanson, H. H., *J. Endocr.*, **51**, 217 (1971).
- ¹⁰ Payne, A. P., and Swanson, H. H., *Physiol. Behav.*, **8**, 687 (1972).
- ¹¹ Vandenbergh, J. G., *Anim. Behav.*, **19**, 589 (1971).
- ¹² Grant, E. C., and MacKintosh, J. H., *Behaviour*, **21**, 246 (1963).
- ¹³ Payne, A. P., and Swanson, H. H., *Behaviour*, **36**, 259 (1970).

Haemoglobin of the Coelacanth

AMONG extant forms of primitive fishes which separated early from the phyletic stock of living vertebrates, the coelacanth, *Latimeria chalumnae*, holds a central position. Although 68 coelacanths have been caught since 1938 very little is known about their physiology and biochemistry. The recent French-British-American expedition to the Comoros captured two *Latimeria* from which we received refrigerated samples of whole blood and packed red cells. Here we describe the respiratory properties of the whole blood and purified haemoglobin and compare these with those of other fishes and tetrapod vertebrates; we also discuss some novel functional properties of *Latimeria* haemoglobin.

Haemoglobin solutions were purified by column chromatography using 'Sephadex G-25'. There was no detectable ATP (measured enzymatically) in the eluted haemoglobin and measurement of extinction at 280 nm showed a single symmetrical peak. Oxygen dissociation curves of phosphate-free and ATP supplemented haemoglobin solutions were determined using the diffusion chamber technique^{1,2}. The temperature coefficient and apparent heat of oxygenation (ΔH) for the HbO₂ binding were calculated. The erythrocyte and haemoglobin values for *Latimeria* seem more like those of the Agnatha and the Chondrichthyes than other bony fishes (Table 1), but critical evaluation of phylogenetic trends must await additional *Latimeria* blood.

Methaemoglobin concentration was 10% of the total haemoglobin, an average value for fish blood³. The oxygen capacity of 4.2% v/v was reduced to 3.7% v/v when equilibrated with 3% CO₂ in air, indicating a moderate Root effect.

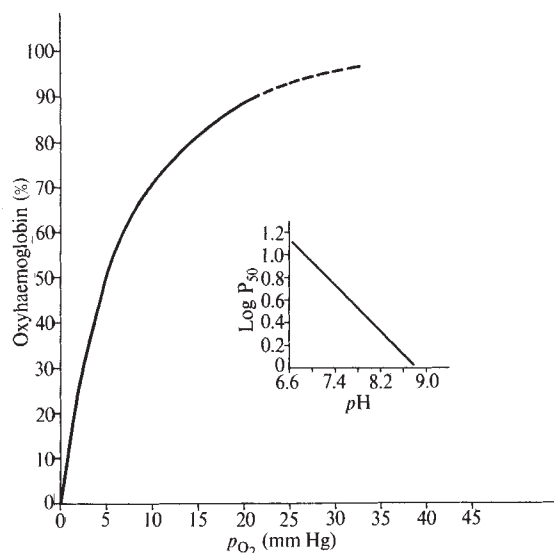


Fig. 1 Oxygen dissociation curve of *Latimeria* whole blood; slope of $\log P_{50}$ — pH plot is Bohr factor—0.5. Temperature 20°C , pH 7.4.

Latimeria whole blood has a high affinity for oxygen (Fig. 1). The P_{50} value was 3.3 mm Hg at pH 7.8 probably near the normal pH at the habitat temperature of 15° – 20°C ^{4,5}. The dissociation curve is hyperbolic ($n=1.25$). The Bohr factor (–0.50) is similar to that of other fish⁶ and human haemoglobins. The buffering capacity of whole blood, expressed as $\Delta HCO_3^- \text{ mM l.}^{-1}/pH$, was 9.0.

Purified haemoglobin solutions revealed a very high HbO_2 affinity with P_{50} of 1.5 mm Hg at pH 7.8 and 20°C . Again the curve was nearly hyperbolic ($n=1.25$). The Bohr factor of purified haemoglobin was similar to both whole blood and unpurified haemolysate (Fig. 2). The temperature sensitivity of the HbO_2 binding (Fig. 3) likewise resembles that for other fish haemoglobins. $\Delta H = -10.4$ kcal/mol Hb is within the typical vertebrate range of –10 to –12 kcal/mol Hb⁷.

A finding not previously reported for other adult vertebrate haemoglobins is that the degree of haem–haem interaction is inversely proportional to pH . Fig. 4 shows the relationship between n -value and pH for whole blood from *Latimeria* 1 and purified Hb-solutions from *Latimeria* 1 and 2.

Riggs⁷ refers to unpublished observations on *Latimeria* haemoglobin showing a Bohr factor of –0.55 between pH 7.0–8.0 which agrees closely with the presently obtained values. A decreasing n value with increasing pH was also noted by Riggs. Mammalian blood, at extreme pH values, and a few other species of fish have been reported to show pH dependent n values, but the effect is generally in the opposite direction. Thus in *Protopterus*⁷, *Raja binoculata*⁸, and the Atlantic tuna, *Thunnus thynnus*⁹ the n value increases with increasing pH in the physiological range. Recently carp haemoglobin has been reported to show a maximum n value at pH 7.0 (ref. 10). An

increase in cooperativity of O_2 binding with decreasing pH occurs for the ammocoetes larvae but not the adult lamprey¹¹. An effect of pH on n would result in increased oxygen delivery to more acid tissues and thus have adaptive value in oxygen transport. The importance of this effect *vis à vis* the Bohr effect in the *Latimeria* must remain unknown until normal blood and tissue pH levels become known.

The importance of organic phosphates as allosteric modifiers of oxygen binding by Hb is now well established for enucleated

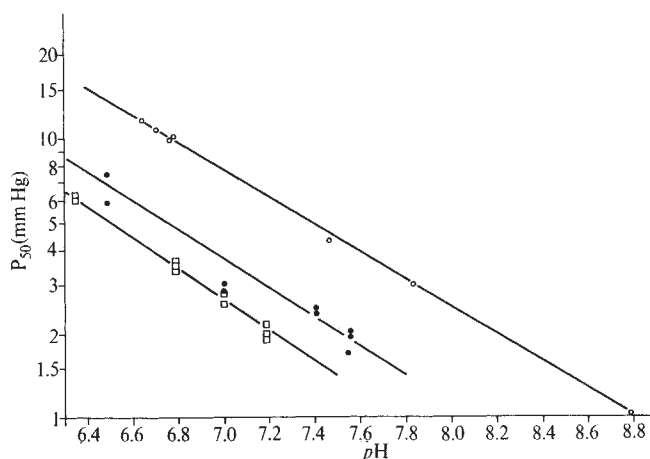


Fig. 2 Influence of pH on P_{50} of *Latimeria* whole blood and haemoglobin solutions. Temperature 20°C . Regression equations: whole blood ($\circ$), $\log P_{50} = 4.40 - 0.51 pH$, $r = -0.99$; haemolysate ($Hb = 0.81$ mM) ($\square$), $\log P_{50} = 4.43 - 0.57 pH$, $r = -0.99$; purified Hb (0.13 mM) ($\bullet$), $\log P_{50} = 3.91 - 0.48 pH$, $r = -0.96$. Phosphate buffers ($I = 0.1$).

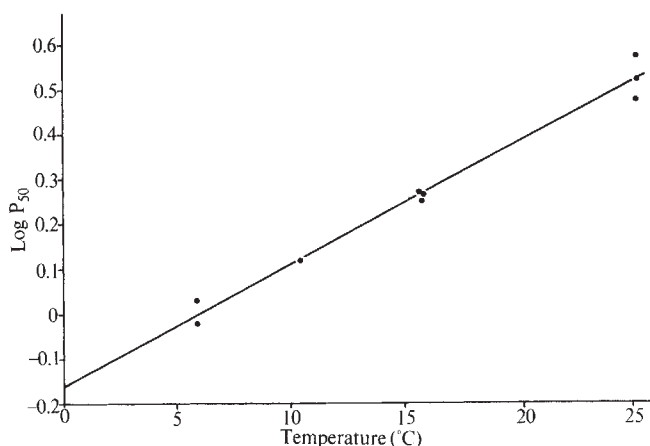


Fig. 3 Effect of temperature on oxygen affinity of *Latimeria* Hb. Phosphate buffer ($I = 0.1$) pH 7.19, $Hb = 0.81$ mM. Regression equation; $\log P_{50} = -0.161 + 0.027 (\text{temp.})$, $r = 0.99$, $\Delta H =$

$$-4.574 \frac{T_1 T_2}{T_2 - T_1} \Delta \log P_{50} = -10.42 \text{ kcal/mol.}$$

Table 1 Erythrocyte and Haemoglobin Values from *Latimeria chalumnae* in Comparison with Average ($\pm$ s.e.m.) Values of other Osteichthyes and Agnatha and Chondrichthyes. Compiled from Data in Altland²⁸

	<i>Latimeria</i> 1	Agnatha 2	Chondrichthyes 9	Osteichthyes 20
Number of species				
Haematocrit %	20.0	22.8 ± 0.65	18.8 ± 2.0	31.4 ± 2.9
Haemoglobin g/100 ml. blood	3.4	5.2 ± 0.6	3.6 ± 0.4	7.3 ± 0.6
Haemoglobin g/100 ml. red cells	17.0	22.8 ± 1.8	19.7 ± 1.4	21.0 ± 1.8
Red cell count millions/ μ l.	0.17	0.24 ± 0.1	0.26 ± 0.04	1.98 ± 0.2
Red cell volume μ m ³	1,163.0	$1,120.0 \pm 410$	798 ± 106.3	184 ± 15.9
Red cell haemoglobin pg	198.0	247.0 ± 71.2	149.9 ± 14.6	40.0 ± 3.9
Red cell dimensions μ m	17.1	20.3 ± 6.1	22.3 ± 1.3	11.9 ± 0.3
	$\times 11.8$	$\times 16.3 \pm 2.0$	$\times 15.1 \pm 1.1$	$\times 8.0 \pm 0.2$

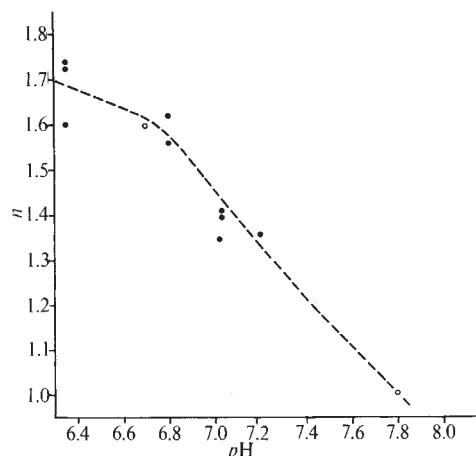


Fig. 4 Influence of pH on haem-haem interaction expressed as "n" of the Hill equation²⁷. Phosphate buffers ($I=0.1$) Hb=0.63 mM. ●, Hb solution; ○, whole blood.

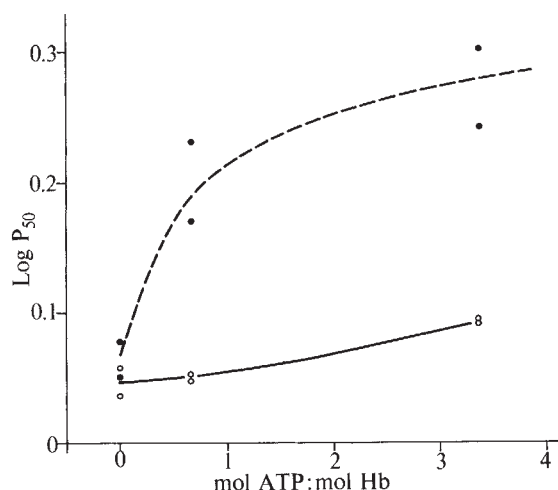


Fig. 5 Effect of ATP on oxygen affinity of *Latimeria* haemoglobin. Tris buffer, ●'s=pH 6.65, ○'s=pH 7.47, Hb=0.18 mM.

red cells of mammals^{12,13} and nucleated red cells of birds¹⁴, amphibians¹⁵ and fishes^{16,17}. The allosteric effect of organic phosphates is absent in the monomeric haemoglobins of cyclostome fishes^{18,19}. The low n value of *Latimeria* haemoglobin with correspondingly low haem-haem interaction promised an interesting target for evaluation of organic phosphate effect on HbO₂ affinity.

The limited volume of whole blood available precluded measurement of red cell organic phosphate concentration. ATP, however, is known to be the major organic phosphate component in red cells of fishes^{16,20}, occurring in concentrations equimolar to haemoglobin. The pronounced effect of ATP on O₂ affinity of purified *Latimeria* Hb is shown in Fig. 5. As in other fishes¹⁶ and mammals²¹ the effect is more pronounced at lower pH.

The idea that a high HbO₂ affinity signifies a phylogenetically primitive condition has been advanced earlier by Wald²² but strongly countered by Manwell¹¹. HbO₂ affinity in fishes shows an indisputable relationship to oxygen availability in the ambient water, with O₂ deficient habitats correlating with higher HbO₂ affinities^{23,24}. The O₂ content of the water at the depth of 200–400 m in the location where *Latimeria* have been caught is not known but studies in other tropical oceans have indicated that O₂ deficient layers are rather common. Whether the exceptionally high O₂ affinity of *Latimeria* haemoglobin is primitive or specialized must hence await further studies. It is evident, however, that if blood and tissue pH are within the normal range for other fishes, oxygen unloading at

the tissue capillaries must occur under extremely hypoxic conditions.

The haemoglobins of lampreys and hagfishes give evidence to theories of haemoglobin evolution based on a monomeric ancestral haemoglobin²⁵. The association of monomeric haemoglobins and subsequent evolution of quaternary structure basic to the Bohr effect and haem-haem interaction is fully manifest in high bony fishes whereas the elasmobranchs²⁶ and chondrosteian fishes (K. J., Hansen and Lenfant, unpublished results), show absent or reduced haem-haem and a low haem-proton interaction. In this scheme *Latimeria* haemoglobin, a tetramer²⁷ with small haem-haem but large haem-proton interaction, is intermediate.

S. C. WOOD
K. JOHANSEN

Department of Zoophysiology,
University of Aarhus,
Aarhus

R. E. WEBER

Netherlands Institute for Sea Research,
Texel

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- ¹ Niesel, W., and Thews, G., *Pflügers Arch. ges. Physiol.*, **273**, 380 (1961).
- ² Sick, H., and Gersonde, K., *European J. Biochem.*, **7**, 273 (1969).
- ³ Cameron, J., *Comp. Biochem. Physiol.*, **40A**, 743 (1971).
- ⁴ Howell, B. J., Baumgardner, F. W., Bondi, K., and Rahn, H., *Amer. J. Physiol.*, **218**, 600 (1970).
- ⁵ Millot, J., *Sci. Amer.*, **34**, 831 (1955).
- ⁶ Sullivan, B., and Riggs, A., in *Respiration and Circulation* (edit. by Altman, P. L., and Dittmer, D. S.), 192 (Federation of American Societies for Experimental Biology, Bethesda, 1971).
- ⁷ Riggs, A., in *Physiology of Fishes* (edit. by Hoar, W. S., and Randall, D. J.), **4**, 209 (Academic Press, New York, 1970).
- ⁸ Manwell, C., *Science*, **128**, 419 (1958).
- ⁹ Rossi-Fanelli, A., and Antonini, E., *Nature*, **188**, 895 (1960).
- ¹⁰ Tan, A. L., De Young, A., and Noble, R. W., *J. Biol. Chem.*, **247**, 2493 (1972).
- ¹¹ Manwell, C., in *Biology of the Myxine* (edit. by Brodal, A., and Fänge, R.) (Oslo University Press, Oslo, 1963).
- ¹² Benesch, R., and Benesch, R. E., *Biochem. Biophys. Res. Comm.*, **26**, 162 (1967).
- ¹³ Chanutin, A., and Curnish, R., *Arch. Biochem. Biophys.*, **121**, 96 (1967).
- ¹⁴ Benesch, R., and Benesch, R. E., *Nature*, **221**, 618 (1969).
- ¹⁵ Wood, S. C., *Respir. Physiol.*, **12**, 53 (1971).
- ¹⁶ Gillen, R. G., and Riggs, A., *Comp. Biochem. Physiol.*, **38B**, 585 (1971).
- ¹⁷ Wood, S. C., and Johansen, K., *Nature*, **237**, 278 (1972).
- ¹⁸ Benesch, R. E., and Benesch, R., *Fed. Proc.*, **29**, 1101 (1970).
- ¹⁹ Johansen, K., Lenfant, C., and Hanson, D., *Comp. Biochem. Physiol.* (in the press).
- ²⁰ Rapoport, S., and Guest, G. M., *J. Biol. Chem.*, **138**, 269 (1941).
- ²¹ Benesch, R. E., Benesch, R., and Yu, C. I., *Biochemistry*, **8**, 2567 (1969).
- ²² Wald, G., in *Modern Trends in Physiology and Biochemistry* (edit. by Baron, E. S. G.) (Academic Press, New York, 1952).
- ²³ Krogh, A., and Leitch, I., *J. Physiol.*, **52**, 288 (1919).
- ²⁴ Johansen, K., and Lenfant, C., in *Oxygen Affinity of Haemoglobin and Red Cell Acid-Base Status* (Munksgaard, Copenhagen, 1972).
- ²⁵ Ingram, V., *Nature*, **189**, 704 (1961).
- ²⁶ Lenfant, C., and Johansen, K., *Respir. Physiol.*, **1**, 13 (1966).
- ²⁷ Weber, R. E., Bol, J. F., Wood, S. C., and Johansen, K., *Arch. Biochem. Biophys.* (in the press).
- ²⁸ Altland, P. D., in *Respiration and Circulation* (edit. by Altman, P. L., and Dittmer, D. S.), 153 (Federation of American Societies for Experimental Biology, Bethesda, 1971).

Susceptibility to Cold in Newborns of Levodopa-treated Rats

LEVODOPA was developed for treating diseases of late maturity^{1,2}, but it is now being administered for diseases that occur during the child-bearing age³. Questions have arisen therefore about its effect on offspring. Observations were made on the offspring of rats treated with levodopa before mating or during gestation. Control mothers were treated with saline.

Sprague-Dawley virgin female rats weighing 200–250 g were caged in groups of three with one male at room temperature. Their mating was timed by the appearance of spermatozoa in the vaginal smear. Levodopa was given by daily gastric instillation. One day prior to estimated delivery, the rats were isolated for observation at 16–25° C. Mortality rates of the offspring during the first 12 hours after birth were recorded.

Inspection of offspring revealed a blue spot, which appeared a few hours after birth, between the scapulae of some newborn rats, with a much higher incidence in the offspring of mothers treated with levodopa during gestation, even in those treated on the first five days only. Levodopa has no effect when administered during the two weeks prior to mating. Most of the young with a blue spot were selectively cannibalized by both treated and untreated mothers and also by foster mothers. The blue spot itself was apparently not the signal for killing, because healthy newborns tattooed with a similar spot were nursed to weaning. Levodopa also increased the spontaneous mortality rate. Dissection revealed extensive subcapsular haemorrhages restricted specifically to the brown fat. Detailed macroscopic and microscopic examinations of the other tissues showed no abnormalities. Haemorrhages did not occur during intra-uterine life (Table 1).

Table 1 Brown Fat Haemorrhage in Twenty-day Rat foetuses from Levodopa-treated Mothers

	% of foetuses with BF haemorrhage	No. of foetuses	No. of litters
Dopa 0.1 mg/g 1–10 day	0	33	3
Dopa 1 mg/g 1–10 day	0	39	4
Dopa 1 mg/g 1–20 day	1	34	3
Control	1	63	6
Total		169	16

Mothers were decapitated and foetuses were examined immediately. Note that haemorrhages were essentially absent during intrauterine life.

The brown fat surrounding the thorax of newborn mammals^{4,5} is rich in both mitochondria⁶ and microvacuolar lipids⁷ and serves as the chief source of body heat^{5,8} early in extra-uterine life^{9–11}.

The brown fat does not become histologically visible until the eighteenth day of gestation¹².

Whether postnatal survival had been jeopardized by impairing the thermogenic contribution of brown fat was tested by placing representative newborns in the cold (10° C) until death (Fig. 1). Survival was reduced markedly in blue spot animals and to a lesser extent in the rest of the offspring of levodopa-treated mothers in comparison with those treated with saline. This suggests that the blue spot is a manifestation of an extreme form of some lesion in brown fat, promoted by levodopa. Histological comparisons were made therefore

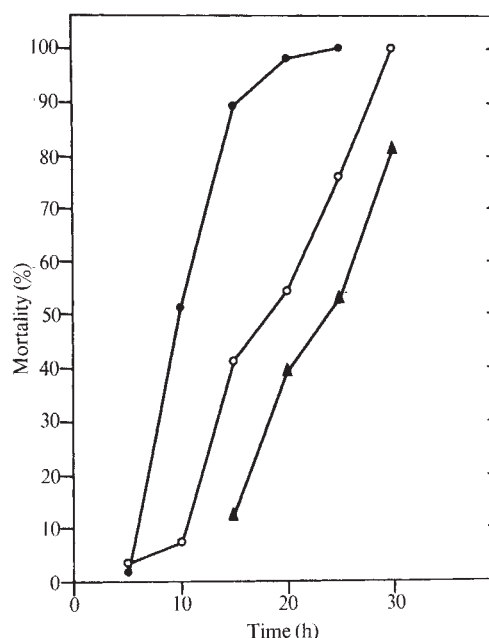


Fig. 1 Newborn rats 2–12 h old were placed in a refrigerator at a constant temperature of 10° C without their mothers. (Group 2, Table 2). Every 3–6 h they were removed for observations lasting 20 min and the survivors were returned to the cold. In six healthy control newborns kept at 22° C without their mothers, death occurred at 54 h. ○, Dopa 20 d; ●, haemorrhage; ▲, control.

	No.	Weight g	50% survey (h)	P
Dopa (22 days) haemorrhage	21	4.930	9.71 ± 2.35	<0.001
Dopa (22 days)	119	5.974	18.70 ± 4.56	<0.001
Control	48	5.927	23.77 ± 6.98	

between (1) control newborns from saline-treated mothers; (2) newborns with a blue spot from levodopa-treated mothers; (3) newborns without a blue spot from treated mothers; and newborns from treated mothers, exposed to the cold until (4) death. Paraffin sections were stained with hematoxylin and eosin and frozen sections were stained for lipids and glycogen. Experimental animals showed marked vasodilation with extensive haemorrhages in the brown fat of newborns in groups (2) and (3), microvascular lipids in the fat were depleted in all experimental animals, but to a greater degree in groups (2) and (3). There were no lesions in other tissues.

Evidence for metabolic interaction between levodopa and manganese^{13–15} has been supported by the work of Cotzias *et al.*¹⁶, who have shown an overlap in their transport systems. Levodopa (1 mg/g/day) was administered during the entire

Table 2 Brown Fat Haemorrhage in Newborn Rats from Levodopa-treated Mothers

Treatment	A % of newborns with BF haemorrhage	B % of offspring with early mortality	A + B	No. of animals	No. of litters
(1) Dopa 1 mg/g 1–5 day	14.7	1.6	16.3	61	5
(2) Dopa 1 mg/g 1–22 day	16.5	7.7	24.2	258	24
(3) Dopa 1 mg/g 1–10 day	14.8	6.5	21.3	129	14
(4) Dopa 0.1 mg/g 1–10 day	12.0	0	12.0	50	6
(5) Dopa 1 mg/g 15 days before pregnancy	3.2	0.7	3.9	140	14
(6) Dopa 1 mg/g 1–22 day MnCl ₂ 0.2 mg/g	7.5	20.9	28.4	172	18
(7) Dopa 1 mg/g 1–22 day MnCl ₂ 0.02 mg/g	3.7	7.5	11.2	53	5
(8) Dopa 1 mg/g 1–22 day Mn-deficient diet (milk)	28	53	81	51	5
Control	2.94	0	2.94	339	34
Total				1,253	125

Brown fat haemorrhage in experimental and control animals; for treatments 1, 2, 3, 4 and 8, $P < 0.001$; for treatment 6, $P < 0.05$; for treatments 5 and 7, P not significant.

pregnancy with and without manganous chloride to the regimen (0.02 mg Mb ²⁺/g/day). The incidence of haemorrhage in brown fat was greatly reduced by the addition of manganous chloride to the regimen (Table 2). The effect of manganese administration could be due to some change in the metabolism of levodopa. Administration of a manganese deficient diet of cow milk with levodopa (1 mg/g/day) markedly increased both incidence of bluespot and mortality of newborns (Table 2).

The experiments show that levodopa administration to rats during pregnancy can damage the brown fat. The selective cannibalization of such offspring by their mothers seems to be an indirect consequence of levodopa administration. Co-administration of manganese appears to have a preventive effect on the damage to brown fat induced by levodopa. These procedures offer a laboratory model for the study of defective thermoregulation in early life.

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ISMAEL MENA*

Medical Research Center,
Brookhaven National Laboratory,
Upton, New York 11973

GILBERTO LÓPEZ
KAZUKO HORIUCHI
HECTOR CROXATTO, jun.

Departments of Nuclear Medicine
and Pathology,
Catholic University,
Santiago.

Received November 22, 1971; finally revised June 16, 1972.

*Reprint requests to I. M.

- ¹ Cotzias, G. C., Papavasiliou, P. S., and Gellene, R., *New Eng. J. Med.*, **280**, 337 (1969).
- ² Mena, I., Court, J., Fuenzalida, S., Papavasiliou, P. S., and Cotzias, G. C., *New Eng. J. Med.*, **282**, 5 (1970).
- ³ Rosenthal, R. K., McDowell, F. H., and Cooper, W., *Neurology*, **22** (1), 1 (1972).
- ⁴ Dawkins, M. J. R., and Hull, D., *Scient. Amer.*, **213**, 62 (1965).
- ⁵ Smith, R. E., and Horwitz, B. A., *Phys. Rev.*, **49** (2), 330 (1969).
- ⁶ Lever, J. D., and Chapell, J. B., *J. Biophys. Biochem. Cytol.*, **4**, 287 (1958).
- ⁷ Menschik, Z., *Anat. Record*, **116**, 439 (1953).
- ⁸ Hsieh, A. C. L., and Carlson, L. D., *Amer. J. Physiol.*, **190**, 243 (1957).
- ⁹ Smith, R. E., *Federation Proc.*, **21**, 221 (1962).
- ¹⁰ Smith, R. E., and Roberts, J. C., *Amer. J. Physiol.*, **206**, 143 (1964).
- ¹¹ Hsieh, A. C. L., Carlson, L. D., and Gray, G., *Amer. J. Physiol.*, **190**, 247 (1957).
- ¹² Sidman, R. L., *Anat. Record*, **124**, 581 (1956).
- ¹³ Papavasiliou, P. S., Miller, S. T., and Cotzias, G. C., *Nature*, **220**, 74 (1968).
- ¹⁴ Cotzias, G. C., Papavasiliou, P. S., Ginos, J., Steck, A., and Düby, S., *Ann. Rev. Med.*, **22**, 305 (1971).
- ¹⁵ Maynard, L. S., and Cotzias, G. C., *J. Biol. Chem.*, **214**, 489 (1955).
- ¹⁶ Cotzias, G. C., Tang, L. C., Miller, S. T., Sladic Simic, D., and Hurlley, L. S., *Science*, **176**, 410 (1972).

Natural Regulation of Numbers in Primitive Human Populations

THERE are several possible mechanisms for the natural regulation of population size in animals¹, such as adjustment of the number of offspring per pair per season, the age of onset of sexual maturity, the percentage of adults reproducing, and the mortality schedules. The regulation of human populations might be achieved in similar ways; three demographic parameters which seem most amenable to adjustment: (i) the age at marriage, (ii) the interval between children, and (iii) the length

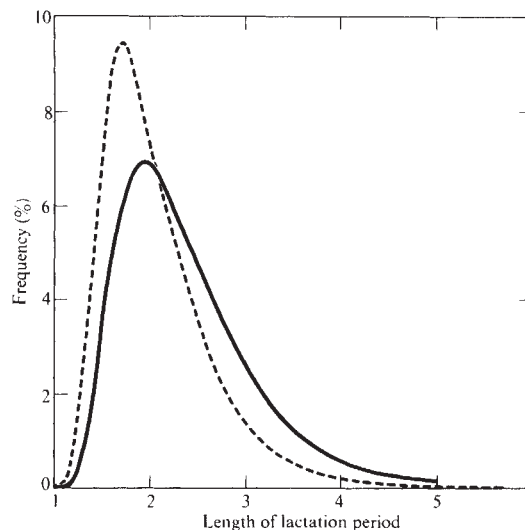


Fig. 1 Distribution of length of lactation period for $N=68$ (---) and $N=45$ (—). The means and variance of the lactation periods are 2.1 and 5.5 and 2.5 and 6.8, respectively.

of life. Here we discuss the way in which adjustment of the childspacing distribution can lead to effective population control.

We used a computer simulation program² constructed to incorporate age-specific fertility, and child-spacing was determined by foetal and infant mortality together with a period of sterility following a birth. This period of post-partum sterility might be due to taboos against coitus during lactation as occur in many populations³⁻⁶, or to a physiological block to conception during lactation. It was possible to adjust the length of this period as a response to population size in the following way. For each 10-yr period we examined the number of marriages N , and adjusted the sterile period for marriages contracted in the next 10 yr. (Specifically we take the sterile period to be distributed as y where $y=(a+bN)x+c$, x being a lognormal variable⁷, and c the minimum possible sterile period.) Fig. 1 shows the distribution of sterile period for $a=0.325$, $b=0.015$ and $c=1.0$ and two values of N .

The mechanism used to relate sterile period and population size is admittedly crude compared with what could be achieved in practice. But, as we shall see, the crude method used is sufficient to control population size, so any more refined method should be even more effective.

Four simulations were made of each of five distinct marriage systems appropriate to primitive man⁸ (random marriage, two-clan exogamy, four-clan exogamy, four-clan cycling, and Kariera). For each system two runs were made in which no link between sterile period and number of marriages was made, and two runs were made with such a link. In every case the method of control proved effective. As an illustration we have plotted, for a four-clan patrilineal exogamous system, the number of marriages per 10-yr period against year. Fig. 2 shows the behaviour for the uncontrolled population, Fig. 3 the controlled population.

In the uncontrolled population the number of marriages per 10-yr period has increased during 600 years to 140, whereas in the controlled population the number of marriages per 10-yr period fluctuates between about 40 and 90 for over 1,200 yr. To achieve this measure of control over population size the mean sterile period has been adjusted only between 2.1 and 2.9 yr. These figures accord well with data on lactation period in the tribes with taboos on coitus during lactation³⁻⁶, and the magnitude of the adjustment seems reasonable. Obviously if it were necessary to vary lactation period between 0 and 8 yr we should be extremely sceptical about its possible use in any populations. Such is not the case here, the mechanism seems both sufficiently strong to prevent population explosion or extinction, and yet sufficiently sensitive not to cause wild

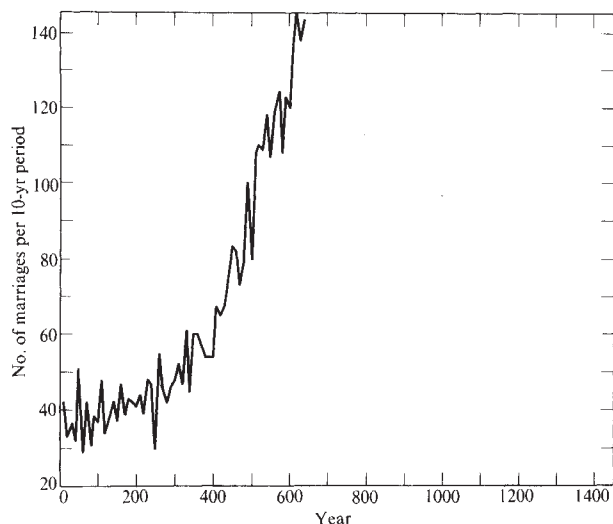


Fig. 2 Number of marriages per 10-yr period for population with unchanging distribution of lactation time.

oscillation, though longer computer runs are desirable to check the latter point.

There remains the question why a tribe should adjust its child-spacing distribution with their numbers. It can, of course, be attributed to a conscious desire to control their numbers, but we should like to suggest an alternative admittedly based on a number of premises requiring further investigation. Consider a population with a constant food supply. When food is scarce (because of a relatively large population), it may be advantageous for lactating women to continue to breast feed their infants for an extended period. This continued lactation may be advantageous for both the infant and the family if the mother is more efficient biochemically at processing the food than her child, a point needing further study. The scarcity of food will thus lead immediately to a reduction in fertility. With a more abundant food supply, that is, a smaller population, an earlier age of weaning will be acceptable, and a consequently higher fertility. This suggested mechanism generates a relationship of the type we have used in our simulation, though not necessarily a linear one like that we have used.

Our arguments do not depend on a constant food supply, nor on an even distribution of this food among families, though we do presuppose a social structure which distributes

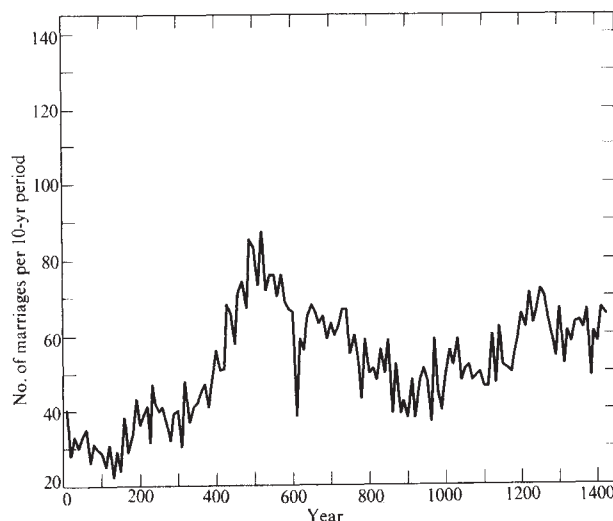


Fig. 3 Number of marriages per 10-yr period for population with distribution of lactation time dependent on population size (see text for exact relationship.)

the food rather than let it be monopolized by a small group. Such uneven distribution as does occur will simply mean that the adjustment has different effects on different sections of the population but does not alter our conclusions.

The acceptance of the existence of a relationship between utilization of food supply and lactation leads us to postulate the importance of an uninterrupted lactation period. We have already pointed out that many tribes have prohibitions on coitus during lactation, and this may have arisen to prevent the interruption of lactation by conception, or by birth. Physiological barriers to conception during lactation would provide an additional safeguard. Such barriers have been discussed by various authors⁹⁻¹¹, and it is clear that the block to pregnancy is at best incomplete. It is for this reason that Birdsall¹² states that "lactation alone would not have provided sufficient spacing of birth to maintain equilibrium" (for population size) ignoring the possibility of taboos. A strong prohibition against coitus during lactation would meet this objection.

One final point should be made. Taboos against coitus during lactation often occurs in tribes practising polygamy, whereas our simulations were run on the assumption of monogamy. The women, however, are essentially the deciding factor with regard to child-spacing in either case, so our results should be valid for polygamous tribes also. The taboo does also exist among fourteen primarily monogamous tribes such as the Bahaya¹³. Our results are directly applicable to these.

M. H. SKOLNICK*

Istituto di Genetica,
Universita di Pavia,
27100 Pavia

C. CANNINGS

Department of Probability and Statistics,
University of Sheffield,
Sheffield S3 7RH

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* Present address: Department of Genetics, Stanford University Medical School, Stanford, California 94305.

- ¹ Wynne-Edwards, V. C., *Animal Dispersion in Relation to Social Behaviour* (Edinburgh, 1962).
- ² Cannings, C., and Skolnick, M. H., *Proc. Fourth Intern. Congr. Human Genetics*, Paris (1971).
- ³ Turnbull, C. M., *The Forest People* (Simon and Schuster, New York, 1961).
- ⁴ Turnbull, C. M., *Bing Wartenstein Symposium* (1970).
- ⁵ Neel, J. V., and Chagnon, N. A., *Proc. US Nat. Acad. Sci.*, **59**, 680 (1968).
- ⁶ Neel, J. V., *Science*, **170**, 815 (1970).
- ⁷ Aitchison, J., and Brown, J. A. C., *The Lognormal Distribution with Special Reference to its Uses in Economics* (Cambridge University Press, London, 1957).
- ⁸ Fox, R., *Kinship and Marriage* (Aldine, Chicago, 1963).
- ⁹ Gioiosa, R. N., *Amer. J. Obstet. Gynecol.*, **70**, 162 (1955).
- ¹⁰ Bonte, M., and Van Balen, H., *J. Biosoc. Sci.*, **1**, 97 (1969).
- ¹¹ Jain, A. K., Hsu, T. C., Freedman, R., and Chang, M. C., *Demography*, **7**, 255 (1970).
- ¹² Birdsall, J. B., in *Man the Hunter* (edit. by Lee, R. B., and Devore, I.) (Aldine, Chicago, 1968).
- ¹³ Richards, A. I., and Reining, P., in *Population and Culture* (edit. by Lorimer, F.) (Unesco, 1954).

A Glutaminase in the Body Wall of Terrestrial Isopods

THE ability of some terrestrial invertebrates to discharge their nitrogen endproduct in the form of gaseous ammonia seems to violate "Needham's rule" which states that owing to the toxicity of ammonia the ammonotelism of aquatic animals has been replaced by ureotelism and uricotelism in terrestrial groups. Both gastropods¹ and isopods² excrete some or most of their N-endproducts as gaseous ammonia. This method would seem to preserve ammonotelism, the "cheapest" way of excreting nitrogen, without its negative corollary, the loss of large amounts of body fluid.

Urea appears to be the major source of NH_3 in gastropods and urease the required catalyst¹. In isopods the concentrations of urea and of urease are insignificant and it has been suggested that NH_3 is created by peroxidative deamination of amino-acids². In *Porcellio scaber* and *Oniscus asellus* I have found high levels of glutamine and glutamate; the presence of an active glutaminase in the body wall makes it seem very likely that glutamine is the dominant source of ammonia in this group of animals. Peculiarities of the enzyme suggest that its activity is controlled by cofactors which in turn are influenced by seasonal variables, physiological features not found in the only glutaminases—of microorganisms, plants and of vertebrates—previously studied⁴.

Isopods were decapitated and eviscerated (gut, hepatopancreas and gonads were removed). 400–500 mg fresh weight of the carcasses were ground with quartz sand in distilled water, or in 0.05 M phosphate buffer, pH 7.0, at a dilution of 1:6. The homogenate was centrifuged at 5,000g for 5 min and the supernatant either used directly or run through a 'Sephadex G-25' column. The latter procedure eliminated unidentified processes of ammonia consumption which cause a lagging in the glutaminase reaction when the crude homogenate is used.

Aliquots of 0.5 ml. of eluant were incubated with 0.5 ml. of 0.1 molar Tris or phosphate buffer, and 0.2 ml. of substrate solution in a shaking incubator at various temperatures. The ammonia produced was trapped by the diffusion method of Sayre and Roberts⁵, in which the assay medium is alkalized in bottles rotating slowly on a wheel, and the concentration was determined by means of a modified phenol-hypochlorite method⁶. The non-enzymatic hydrolysis of glutamine during the diffusion process was restricted to low levels by operating the wheel in a refrigerated box at about 0° C.

The reaction rate remained constant for at least 1 h and the

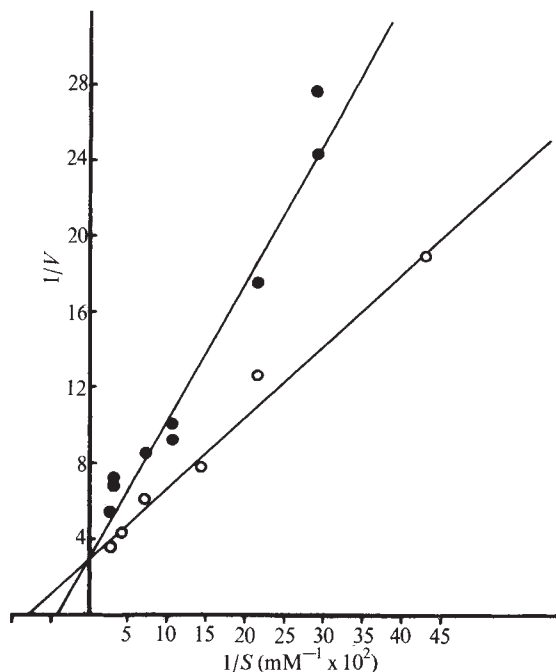


Fig. 2 Lineweaver-Burk plot of the glutaminase activity in homogenates of the body wall of *P. scaber*. The crude homogenate was run through a 'Sephadex G-25' column. At 20° C six samples in July had about twice the apparent substrate affinity than nine samples in August. ●, $K_m = 25 \times 10^{-3} \text{ M l.}^{-1}$ 10–18.8; ○, $K_m = 13.4 \times 10^{-3} \text{ M l.}^{-1}$ 11–12.7.

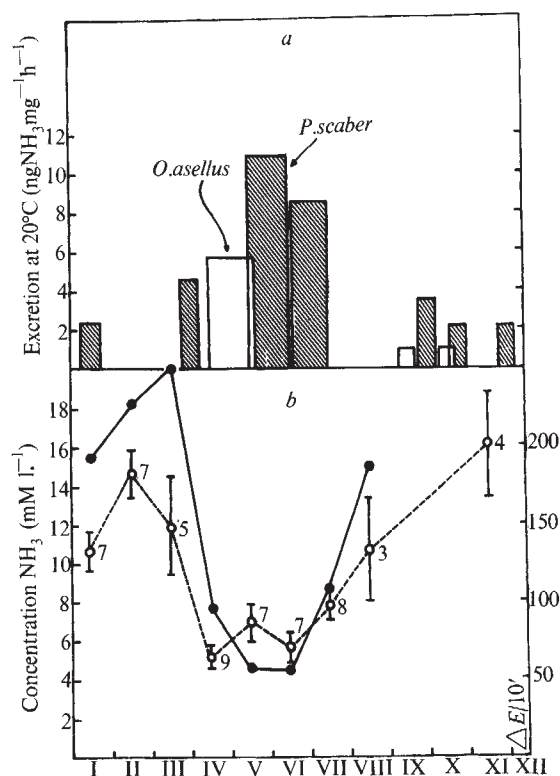


Fig. 1 Seasonal fluctuations (1970) of variables. a, Ammonia excretion of two species of isopods, each column represents a group of experimental animals, all from the vicinity of Innsbruck, Austria. b, ○, ammonia content of body wall of *P. scaber* in mM/l. tissue water; ●, relative activity of glutaminase in raw homogenates of body wall at a substrate concentration of 22.8 mM/l. at 30° C. Means, s.e. and *n* are indicated. A formally similar relationship was obtained in 1971, when enzyme activity was expressed per mg protein, and the raw homogenate run through a 'Sephadex G-25' column.

enzyme was not activated by phosphate or by pyruvic acid. The reaction is pH-dependent and shows a peak at pH 7.8 with a broad shoulder towards pH 7.0. At pH 6.0 the reaction velocity was about one-tenth that at pH 7.8. At high substrate levels (around 20 mM l.⁻¹) the enzyme was 2.5–5 times more active in winter than in summer, the drop taking place at different times in spring, according to the provenance of the animals. The seasonal difference in glutaminase activity was directly proportional to the ammonia concentration of the body wall, and inversely proportional to the amount of ammonia excreted by the animals in gaseous form (Fig. 1). This finding poses several questions.

The high activity of the enzyme in winter may be connected with the burst-like mode of ammonia excretion during this season⁶. In one of these bursts more ammonia may be released per time unit (up to 2 $\mu\text{M g}^{-1}$ fresh weight/h at 20° C) than is ever released at one time in summer, although when measured over longer periods and at the same temperature the average ammonia production is up to five times higher in summer than in winter². In order to maintain the burst-like mode of ammonia excretion at the low temperatures prevailing in winter and spring, a higher concentration of the enzyme, or a more efficient version of it, would be required. At 20° C and at the physiological substrate concentration of 10 mM l.⁻¹ the glutaminase of the body wall produces up to 5 $\mu\text{M NH}_3 \text{ g}^{-1}$ fresh weight/h *in vitro* and thus is capable of releasing ammonia in excess of the rate measured *in vivo*.

Whether the proportionality of glutaminase activity and ammonia content of the body wall reflects a causal relationship remains to be seen. It is possible that the high enzyme activity and the burst-like release of gaseous ammonia in winter calls for an increase in the storing capacity for dissolved ammonia (maximum value measured: 20 mM l.⁻¹ tissue water). At any rate, both in summer and winter ammonia could serve as a proton acceptor in the cuticular matrix to create an alkaline environment for the deposition of calcium carbonate. Speeg and Campbell¹ have discussed such a model for the extrapallial fluid of gastropods, and it may be indicative of the mechanisms involved that both groups of terrestrial invertebrates that are capable of discharging part or all of their nitrogen endproduct

as gaseous ammonia have large stores of CaCO_3 in the outer shell of the body. Possession of haemocyanin is another characteristic common to both gastropods and isopods. Perhaps this respiratory pigment is optimally adjusted for operation in a medium rich in calcium⁷ as well as in the alkaline milieu required for the maintenance of a gradient along which ammonia can diffuse from blood to tissues¹.

The molecular causes of the seasonal variation in glutaminase activity are not yet clear, mainly because the membrane-bound enzyme has so far withstood attempts to solubilize it without too great a reduction in its activity. In summer it proved to be easier to obtain reproducible curves with the 'G-25' treated homogenate than in winter. A month-by-month check of enzyme activity suggests that at this time of year changes in enzyme activity are due to the interference of a competitive inhibitor (Fig. 2). However, the increase in activity observed at high substrate concentrations in winter is not accompanied by an increase in substrate affinity. The enzyme appears to be part of a complex system that adjusts the rate of ammonia production and the storing capability of terrestrial isopods to the physiological state of the organism which, in turn, is controlled by seasonal variables.

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W. WIESER

Institut für Zoophysologie der Universität,
Innsbruck

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¹ Speeg, K. V., and Campbell, J. W., *Amer. J. Physiol.*, **214**, 1392 (1968).

² Wieser, W., and Schweizer, G., *J. Exp. Biol.*, **52**, 267 (1970).

³ Hartenstein, R., *Amer. Zoologist*, **8**, 507 (1968).

⁴ Meister, A., *Biochemistry of the Amino Acids*, vols. 1 and 2 (Academic Press, New York and London, 1965).

⁵ Sayre, F. W., and Roberts, E., *J. Biol. Chem.*, **233**, 1128 (1958).

⁶ Wieser, W., Schweizer, G., and Hartenstein, R., *Oecologia*, **3**, 390 (1969).

⁷ Burton, R. F., *Comp. Biochem. Physiol.*, **41A**, 555 (1972).

Possible Adverse Effect of Protozoa on Effluent Purification Systems

EFFLUENT purifications systems based on the "activated sludge system" contain a naturally occurring population of micro-organisms which can be represented as a continuous-flow culture of either chemostat or plug-flow type. In the ideal chemostat type the culture is completely mixed, whereas in the ideal plug-flow type it flows through the vessel without mixing. Both types of system are used, but the choice seems arbitrary although recent theory suggests that the chemostat system is better¹. In view of the large capital sums now being invested in sewage disposal, the arbitrary design of the activated sludge process² calls for urgent investigation. A case in point is the effect of protozoa on the process.

An effluent is biologically purified by the utilization of the soluble waste as a nutrient or substrate for microbial growth and metabolism. It is assumed that bacteria are the principal agents of soluble waste removal. There are also protozoa which consume the bacteria and thereby contribute to clarification of the effluent. Canale³ and Curds⁴ have analysed the relations of the bacterial prey and the protozoan predator in a chemostat process, and show that the three variables, prey, predator and nutrient (sewage) concentrations, may either approach true steady states or oscillate continuously, depending on the microbial growth constants and the dilution rate of the system. In Fig. 1 steady state values have been plotted as a function of dilution rate using growth constants feasible for protozoa as predators and bacteria as prey. Similar results have been obtained by Curds⁴, using different values for the microbial growth constants. The protozoa are washed out of the system if the dilution rate is above the critical value (0.39 h^{-1}) up to which value, in steady states, the specific growth

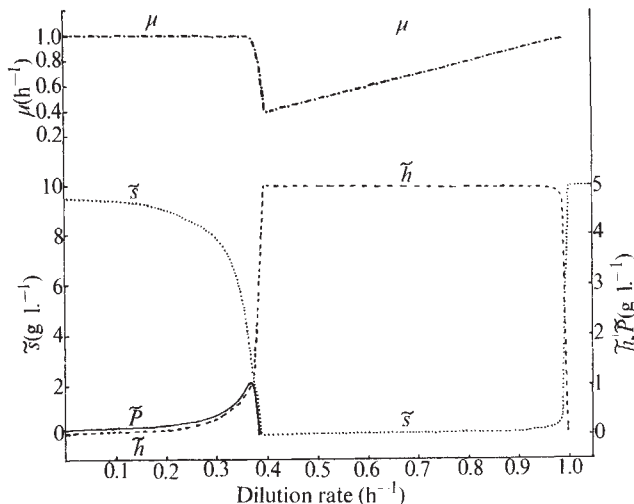


Fig. 1 Steady state values of parameters of a prey and predator culture (for example, bacteria and protozoa) in a chemostat. The fixed parameters were: $\mu_m = 1.0 \text{ h}^{-1}$; $K_s = 0.01 \text{ g l}^{-1}$; $\lambda_m = 0.4 \text{ h}^{-1}$; $K_h = 0.10 \text{ g l}^{-1}$; $Y = 0.5 \text{ g prey g}^{-1} \text{ growth-limiting nutrient}$; $W = 0.5 \text{ g predator g}^{-1} \text{ prey}$; $s_r = 10.0 \text{ g l}^{-1}$. The variables are: s = concentration of growth-limiting nutrient for bacteria; h = concentration of bacteria; p = concentration of protozoa; μ = specific growth rate of bacteria; D = dilution rate. (The tilde signifies a steady state value.) The equations for the system are: $\mu = \mu_m s / (s + K_s)$; $\lambda = \lambda_m h / (h + K_h)$; $dp/dt = (\lambda - D)p$; $dh/dt = (\mu - D)h - \lambda p / W$; $ds/dt = D(s_r - s) - \mu h / Y$. In the steady state $dp/dt = dh/dt = ds/dt = 0$.

rate of the protozoa is equal to the dilution rate. Above the critical dilution rate of the protozoa, only bacteria remain and nutrient utilization is practically complete until near the critical dilution rate for the bacteria (1.0 h^{-1}). It is surprising that when the protozoa are present, the nutrient concentration is high except at dilution rates approaching the critical dilution rate of the protozoa, indicating that predation decreases the number of bacteria in the system, and diminishes the rate of nutrient consumption. Thus it seems that in the presence of a protozoan predator the efficiency of waste (nutrient) removal is significantly less than is potentially possible.

This analysis leads to the conclusion that efficient waste removal combined with subsequent protozoa development may be achieved by two chemostats of differing volumes in series. In the first vessel, of relatively small volume (V_1), the flow rate (f) could be adjusted so that the dilution rate (f/V_1) was higher than the maximum specific growth rate of the protozoa population thus eliminating predatory behaviour and allowing nutrient utilization by the bacteria to achieve its full potential. In the second vessel, of larger volume (V_2), the dilution rate (f/V_2) would be less than the maximum specific growth rate of the protozoa and predation on the incoming bacteria would take place. Using the values of Fig. 1, nutrient utilization would be almost complete with a dilution rate of 0.9 h^{-1} . In the second stage with a dilution rate of 0.3 h^{-1} , the protozoa would consume almost all the bacteria. Hence with a total residence time of 4.4 h in the two-stage process, the nutrient to protozoa conversion would be about 99% efficient. Thus a two-stage system would have the advantages given by the presence of protozoa in an effluent purification system combined with a considerably more efficient mechanism for removal of the waste material.

S. J. PIRT
M. J. BAZIN

Department of Microbiology,
Queen Elizabeth College,
London W8 7AH

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¹ Abson, J. W., and Clarke, E. I., *Proc. Biochem.*, **6**, 15 (1971).

² Pirt, S. J., *Association of River Authorities Year Book*, 119 (1971).

³ Canale, R. P., *Biotech. Bioeng.*, **12**, 353 (1970).

⁴ Curds, C. R., *Water Research*, **5**, 793 (1971).

BOOK REVIEWS

Darwin's Critics

Charles Darwin: The Years of Controversy—The Origin of Species and its Critics, 1859–82. By Peter J. Vorzimmer. Pp. xix+300. (University of London: London, 1972.) £4.40.

IT might have been said of Darwin, as it was of Newton, that he was so happy in his conjectures that he seemed to know more than he could possibly have any means of proving. Newton, however, could provide the proof when required, whereas it was Darwin's misfortune that he could not, for reasons that are made clear by Professor Vorzimmer in this well documented and scholarly analysis of the controversy that developed around the theory of natural selection. The laws of the Newtonian universe had a basic logical and mathematical simplicity, whereas the natural world encompassed by Darwin included complex biological phenomena which could not, during his lifetime, be subjected to mathematical treatment. Particularly damaging, as we can now see, was current ignorance regarding the mechanism of inheritance and the nature and origin of small variations.

Vorzimmer, drawing on new material, including Darwin's annotated reprint collection in the Botany School Library at Cambridge, traces the steps by which certain inconsistencies were introduced into the successive revisions of *The Origin of Species* and shows how these were then seized upon by Darwin's critics with what appears, in retrospect, to be an ill-judged and at times distasteful enthusiasm. Mivart, for example, who was amongst the earliest supporters of the theory of natural selection, later found that his religious views were incompatible with a more general belief in evolution. The result was an ideological estrangement in which a "vituperative attitude" was fortified by dialectic skill founded on his previous legal training.

Vorzimmer argues that what exposed Darwin to this and other attacks was the intellectual honesty with which he

sought the facts that he needed to justify his arguments. Unfortunately, the facts were not always there, and so, rather than ignore the consequent difficulties, he adopted assumptions which could not be consistently subsumed within a single theory. Nevertheless, the sixth and final edition of the *Origin* showed him maintaining his stand against his critics with renewed firmness, but the strain was proving too severe, and he retired from the controversy during his closing years to find relief in botanical studies.

The picture suggested by Vorzimmer, of an elderly Darwin badgered into a state of frustrating confusion, is surely too highly coloured, for he did, after all, live to see wide acceptance of much of his thinking. But in the "Epilogue" to this fascinating study there is a well-judged tribute to the magnitude of Darwin's achievement, and to the superbly consistent faith with which he held to the essentials of his position throughout a momentous intellectual struggle to which we all owe so much.

E. J. W. BARRINGTON

Organic Metaphor

Organic Form: The Life of an Idea. Edited by G. S. Rousseau. Pp. xii+109. (Routledge and Kegan Paul: London and Boston, June 1972.) £2.

ALTHOUGH the terms "organic form" and "organic unity" have long had a cherished place in the terminology of aestheticians, their popularity is not always accompanied by close definition or examination. Often they serve a vaguer, more rhetorical purpose, guarding works of art against premature classification and artists against mechanical rules of judgment. Indeed, the issues raised by their use are so wide-ranging that evasiveness is not unforgivable; even now, it may be that the topic is best approached by a convergence of brief studies, as in this small book, which consists of lectures given by specialists

in three different fields (G. N. Orsini, Philip C. Ritterbush and W. K. Wimsatt) to a section of the annual Modern Language Association meetings, along with an extremely useful bibliography, contributed by the editor.

In the first lecture G. N. Orsini draws attention to the early appearance of the concept in ancient philosophy, as when, in Plato's *Phaedrus*, Socrates declares that every discourse "must be composed like a living being, with a body of its own, as it were, so as not to be headless or footless, but to have members and a middle arranged in a fitting relation to each other and to the whole." Unfortunately it is not clear how seriously Socrates took his metaphor. (As Wimsatt trenchantly asks later, "What is the stomach of a work of art?") Nor does the paper itself offer a satisfactory definition of the basic terms involved. It begins by quoting approvingly Hespers's criterion of "unification":

The unified object should contain within itself a large number of diverse elements, each of which contributes to the total integration of the unified whole, so that there is no confusion despite the disparate elements within the object. . . . In the unified object everything that is necessary is there and nothing that is necessary is not there.

This, however, would surely apply as much to a clock as to a camel. It is hard to see how one can have a satisfactory concept of "organic" unity which does not contain some reference to phenomena associated specifically with life.

Philip C. Ritterbush's paper (taken partly from his interesting book *The Art of Organic Forms*) traces the recent development of the idea. Artistically, the need for it may be seen to have resulted from the breakdown of earlier theoretical justifications for art—as in religiously-authorized ritual or established convention. When such bases come to be questioned, it was natural to look for the authority of art first in its relationship to observable nature—and

then (in face of certain obvious difficulties) to declare that the work of art was something more than a "mirror" to the world, its function differing from that of diagrammatic or exact representation as an organism differs from a machine.

As a mode of distinction (and even of polemic) the point was a fair one, but it leaves us asking how far the image may legitimately be pursued. Certain German *naturphilosophen* took it, perhaps, to its logical conclusion by their implicit assumption that the systematized introspections and generalizations of philosophers concerning possible correspondences between the individual human organism and the external world might provide a scientifically verifiable account of natural phenomena. In practice, of course, such accounts were too far from the observable facts of nature to be of much permanent value—even Goethe's *Metamorphosis of Plants*, as Ritterbush points out, showing little concern for the material processes by which growth took place. At best, it might be said that their desire to find a view of nature that reconciles the human being to the universe is a permanent, if strictly subordinate, factor in much scientific research.

At a less ambitious level, the concept has been of value in both the arts and sciences. As one follows Ritterbush's account, however, certain ambiguities become discernible. "Organic form" can be of at least three types: it may be a relatively stable form, created by an organism and continuing to exist after that organism's death or withdrawal (the nautilus shell and the honeycomb provide obvious and classical examples); or it may be the form of a whole, living organism, caught at a single moment of its existence; or it may be the "total" form, in time and space, of an organism during a long period of development. Normally, I suppose, biologists aim to seize the form of an organism in this third sense (D'Arcy Thompson's remark, "Morphology is not only a study of material things and of the forms of material things, but has its dynamic aspect, under which we deal with the interpretation, in terms of force, of the operations of Energy" comes to mind) but it is noteworthy that the examples of scientific work on organic form given by Ritterbush tend to fall into the first and second categories.

This may simply be a reflexion of Ritterbush's special interest in the visual arts, of course; and no doubt the ambiguity I have mentioned raises few problems for practising biologists. When the concept of organic unity is transferred to art, on the other hand, the ambiguity becomes more crucial. A poem may be regarded as the isolable product of the poet; or it may be read in conjunction with a sense that it expresses the mind and experience of the poet

and/or reader at a particular moment in time; or one may try to contemplate such interplay over a long period, in terms of "organic growth". For Professor Wimsatt there is no problem. With commonsense urbanity he cuts the knot, declaring roundly, "It is only to the finished product that we can apply the concept of organic unity." He also argues that to speak of a poem as an organism in any literal sense is absurd. What he works for, instead, is the doctrine of "an exceedingly subtle, intimate, manifold (and hence dramatic and imaginative) 'interanimation' of parts in a poem." It is an attractive formula, but also a shade elusive, suggesting a desire to reap the rhetorical benefits of organic imagery without actual commitment to organic metaphor. One would like to see such potential objections more clearly faced.

Although Wimsatt's initial distinction makes his discussion more manageable, moreover, it also shuts off some of the more rewarding areas of the topic. Those critics who have found the concept most useful have always, I think, been interested in process as well as product. When they speak of a poem as having organic form they are making a comment not simply about the nature of the poem but about the nature of the poet in making it and the state of mind induced in the receptive reader. Wimsatt advances the (confessedly) "heretical" view that a good chess problem, "viewed according to the idealist organicist norm, has a more fully determined and hence more perfect structure than even a sonnet by Shakespeare." But this is precisely where the full organic metaphor might come to the critic's aid, suggesting the different sensory modes by which a poem communicates—bringing, as one might say (changing Coleridge's terminology slightly) "the whole psyche of man" into operation.

Coleridge, of course, is a crucial figure in the whole affair, for he was largely influential in introducing the concept to English readers. His contribution still tends to be underestimated, drawing partly as it did on an English tradition which differed in certain respects from that of the Germans. Both Ritterbush and Wimsatt, for example, cite Erasmus Darwin's whimsical *Botanic Garden* as an example of poetry which is, so to speak, "aware of the organic", but neither mentions his *Zoönomia*, a more intelligent and, I believe, a more seminal discussion. These matters are too intricate for a short review, however; it is enough to say that anyone who is interested in the topic as a whole, whether from the point of view of science or of the arts, will find much to quicken interest, and many references to follow, in this stimulating volume.

JOHN BEER

Hungarian Research in Crop Production

The First Twenty Years of Martonvasar. Edited by S. Rajki. Prepared for the press by G. Pal. Pp. 329+15 plates. (Agricultural Research Institute of the Hungarian Academy of Science: Martonvasar, 1972.) \$16.00.

A SCIENTIFIC council set up by the Government in 1948 assessed existing facilities for research in Hungary and the need for new institutes. Plans were made for a plant production research institute at Martonvasar to combine practical and theoretical work on genetics, plant breeding, physiology and seed production. The institute began with two workers in 1949, had 19 a year later and now has about 40 scientists. The main developments in the programme were in the 1950s when a large experimental farm was also acquired. Martonvasar, along with other agricultural research institutes, was placed under the Hungarian Academy of Sciences. Successes in the 1950s included the adaptation and development of American methods for producing hybrid maize seed and the selection of new varieties of wheat. The institute claims an important share in the improvement of the yields of Hungary's two main crops—wheat and maize; yields of both have practically doubled since the end of the war, recent peak averages have been 27q/ha of wheat and 38q/ha of maize.

This book has 3 texts—in English, Hungarian and Russian. The director (Dr. Sandor Rajki) described the development of the institute and its experimental farm; publications by members of the staff are listed. Present work is described in separate chapters on: Plant Physiology and Biochemistry (L. Gaspar); Plant Genetics (S. Rajki); Plant Breeding (I. Kovacs) and Plant Production (B. Györfy). The book will be valuable to those interested in the development of agricultural research in Hungary since 1950, to plant breeders, and, obviously, to scientists who may plan to visit Hungary. The account (by A. Kuti) of the institute's experimental farm (of 572 ha) is an interesting example of the practice and economics of a large farming unit.

G. W. COOKE

Experiments on Stress

Urban Stress: Experiments on Noise and Social Stressors. By David C. Glass and Jerome E. Singer. Pp. xiii+182. (Academic: New York and London, March 1972.) \$8.75.

THIS book has a most inappropriate title. One might expect it to cover a variety of the conditions met in modern

cities, to discuss the effects which they may have after prolonged periods, and perhaps to be of interest and relevance to the general public rather than to specialists. In fact however the book discusses short term laboratory experiments, almost exclusively using loud noise rather than any other source of stress, and it would be most unwise to draw from it general conclusions about urban life.

To the specialist, the experiments are of interest and importance. Briefly, Glass and Singer can show statistically significant changes in human behaviour when the people concerned have been exposed experimentally to noise, not during the period of measurement, but during a preceding period. The effects are greater if the noise is unpredictable in time of arrival, and are less if the subject has an emergency button which he believes will stop the noise when he cannot stand it any longer. Thus the measurable effects seem to come from the feeling of inability to control the situation rather than from an automatic and mechanical effect of subjection to noise. This interpretation is confirmed by smaller scale experiments giving similar results but using electric shock rather than noise: and other studies in which the person tested had previously been exposed to a clash with bureaucracy requiring intensive form filling, or an arbitrary discrimination in which he learned that he was to be paid less for his services than a member of the opposite sex would have been. All these conditions produced changes in the performance of psychological tests: these changes might fairly be described as indicating lack of enthusiasm for the experiment, as compared with the state of people who had not met the stressful conditions.

These results are important to specialists because they show both the importance of the social conditions of an experiment upon the results, and also that these effects may persist from one experimental trial into the next, and thus upset comparisons between experimental and control conditions. The methods of measurement used are also very interesting: it is to be hoped that both the techniques and results will be taken into account by future workers in this field. The results are not yet at a stage when non-specialists can go out and apply them to the problems of contemporary cities, however. This is because the stresses applied, the methods of measurement, and the social context are all open to question as applying to real urban situations.

The noise used in the majority of experiments, for example, consisted of blast, the energy of which was an order of magnitude greater than the level produced by jet aircraft at the noisiest position around London Airport. If

there were any city in the world where such levels existed, one would not need psychological evidence to get them stopped, because they would certainly be a substantial hazard to the hearing of anybody in range. Electric shock equally is not a common accompaniment of life in Tower Hamlets. Discrimination and exposure to arbitrary authority are: but unfortunately it is an optimistic assumption that those conditions are new and peculiar to urban life.

So far as methods of measurement are concerned, the problem lies in interpretation. One of the scores used by Glass and Singer is the time spent trying to solve insoluble problems: people who have been exposed to noise or shock give up such problems sooner. But is this a bad thing, or a good one? They also performed less accurately at a task of reading proof: we are not told whether they did the task at the same speed. Which matters most, the speed or the accuracy of an arbitrary routine task? Without much closer investigation, one could not say whether the effects of noise and shock are desirable or undesirable. From a lay and unscientific view, no doubt we may think the latter: but more work is needed to establish it.

From the point of view of social context, these experiments involved the deliberate manipulation, and at times deception and mistreatment, of the people tested by the psychologists involved. It is not clear that the same effects would be produced by the impersonal frustrations experienced in a large city, and one of the key questions is the extent to which this is so and may be prevented. It may be plausible that the commuter will take out on his secretary the discomforts produced by his overcrowded train: but this kind of experiment shows only that he will take out on the experimenter the discomforts induced by the experimenter, and that is not the same thing.

These comments do not of course detract from the value of these experiments to the specialist: but the general reader should beware of drawing conclusions from them. Both the public and the professional psychologist still await studies of prolonged exposure to realistic urban conditions, using methods of measurement which have unambiguous validity. DONALD BROADBENT

Nuclear Curate's Eggs

Elementary Particles. By I. S. Hughes. Pp. 254. (Penguin: Harmondsworth, Middlesex, 1972.) £2.50.

The Atomic Nucleus. By J. M. Reid. Pp. 269. (Penguin: Harmondsworth, Middlesex, 1972.) £2.35.

THESE two books are part of the physics section of the *Penguin Library*

of the *Physical Sciences* under the general editorship of Professor N. Feather. Together with W. M. Gibson's *Nuclear Reactions* they dispose of nuclear physics. The series as a whole is aimed at the first degree candidate; these three volumes catch him in his final year.

Dr Reid's book is a conventional and quite reasonably up-to-date curate's egg (it has shape-isomers and the current speculations about super-heavy elements). Unfortunately its conventionality requires it to sacrifice a lot of space to detailed treatments of matters that are either not nuclear at all (for example, diatomic molecules) or are to be found *ad nauseam* elsewhere (for example, one-dimensional barrier problems and other standard quantum mechanical results) or are only nuclear by tradition (for example, nuclear magnetic resonance and atomic beams); it cannot afford to do this within its strictly limited confines: the real nuclear physics gets dreadfully squeezed, many important topics are just not there and there is, overall, too much repulsive analytical stuff that is not subsequently used and too many rather breathless nods towards the real physics. This must be frustrating for the student and is a pity because Dr Reid clearly has a good feel for the kind of things that should go into an exciting nuclear physics course; he just cannot find the space to get to the heart of the matter. Why write such a book as this? It obviously "had" to be written because the series of which it is a part demanded it irrespective of whether or not adequate texts already existed elsewhere. Dr Reid either chose, or was compelled by editorial policy, to make his text "complete", which was also a pity. If there are to be more undergraduate texts on nuclear structure, please let them concentrate on concepts and insights and not go yet once more over the whole tedious and completely non-nuclear business of just how similar are the level sequences of the square well and the harmonic oscillator, the multipole expansion of the electrostatic potential, the spherical potential well and, O God, O Montreal, the behaviour of atomic systems in magnetic fields.

Dr Hughes's kettle of fish is different and more palatable but may even be held to err in the opposite sense. Dr Hughes has the advantage that his topic is less beaten into the ground by predecessors; and his treatment is at a higher level than that of Dr Reid, which gives him more flexibility. The book is packed with actual experimental data, attractively presented and pedagogically appealing, alternating with meaty but purely *anschaulich* theory that often contents itself with bald assertion. Diametrically opposite to Dr Reid in the

pedagogical premise of his text, Dr Hughes derives almost nothing and therefore demands either that the student take a great deal on trust or that he spend a great deal of time on background material. My view of the matter is that Dr Hughes, less conventional by far, is nearer the mark of what our undergraduate teaching should be. We should try, above all else, to get over the physics sense, indicating clearly what is rigorously derivable and what is not, but eschewing detail, indicating sources for the appropriately involved student but not compelling him to digest extensive and soul destroying formalism that in the first place rests on unphysical approximations and in the second place does not lead to applications that carry his understanding further forward.

Both books are nicely and clearly printed and attractively laid out but the photographs (in Dr Hughes's book) are very sooty.

D. H. WILKINSON

Early Medical Books

Catalogue of Medical Books in Manchester University Library 1480-1700. Compiled by E. M. Parkinson, assisted by A. E. Lumb. Pp. 399. (Published on behalf of the Manchester University Library and the Manchester Medical Society.) (Manchester University: Manchester, May 1972.) £15.

MANCHESTER is or was famed for its cotton mills, its football-teams, its Ship Canal, its Free Trade Hall, its university, its orchestra, its rivalry with Liverpool (and jealousy of London), as well as for its libraries of which John Rylands is the jewel that outshines all others: a rather less sparkling stone, by comparison, being the library now so well catalogued in part by Mrs Parkinson and her aides.

The core of this valuable collection was put together by a few early members of the Medical Society (founded 1834) and was deposited with the University in 1870. Since 1930 it has been administered, with accretions to it, as a part of the University Library and today it stands as probably the best of its kind in the English provinces. These basic facts are mentioned by the University Librarian in a preface in which he pays due credit to the chief benefactors of the past (for example, Christie, Parkinson, Bullock, Arnold). If we may be allowed one quibble, however, it seems a little unfortunate that the preface fails to acknowledge the short-title catalogue to the Society's library published in 1890, the more so as it is bound to retain its usefulness until such time as a promised volume appears covering the 18th and

19th centuries. The preface might also have usefully identified those subjects in which the library is particularly strong (for example, deaf and dumb) and, if nothing else, its earliest book (Guy de Chauliac, *Chirurgia*, 1480). Looking to the future, it is good to learn that the pre-1701 medical books at Rylands will be included in a forthcoming catalogue of the University's History of Science collections.

The bibliographical and production standards reached in this volume of 2,685 entries are very high indeed. Titles and imprints are given fully; cross-references are more than adequate; very few errors of transcription occur; contents of collective volumes are listed at great length (for example, Le Clerc's *Bibliotheca anatomica*, 1685); whilst forming a useful adjunct are indexes of subjects (selective), places, printers and publishers.

Most of the big names are represented. Anyone studying the Renaissance, for example, will be pleased to find Vesalius, Champier, Estienne, Cardano, as well as good crops of Galen (forty-three items) and Hippocrates (fifty-three) and the expected herbals by Dioscorides, Fuchs, Gerard, Parkinson, Dodoens, and Mattioli. For the 17th century there are ten Boyle items, thirty-six by Thomas Bartholinus, nine by Harvey (though not the 1628 *De Motu*), sixteen by Helmont, eleven by Salmon, sixteen by Glauber, Whistler on the rickets (1645), Glisson on the liver (1654), and much else besides. The index reveals only one book printed at Cambridge as against nineteen at Oxford, three at Prague, and two at Edinburgh—with Amsterdam, Basle, Frankfurt, Leyden, London, Paris and Venice taking the greatest number of entries.

The compilers are to be congratulated on a splendid achievement.

E. GASKELL

Antique Instruments

Scientific Instruments of the Seventeenth and Eighteenth Centuries and their Makers. By M. Daumas. Pp. 361+142 Plates. (B. T. Batsford: London, 1972.) £10.

It must be made clear from the beginning that this is a translation of a book originally published in Paris in 1953, a fact nowhere stated in this English edition. It is also appropriate to point out that the French paperback edition is still available, and can be bought for 26 francs.

There are a number of reasons why the historian of science would prefer

to use the French version: the name index includes dates when known, the notes and references are in their proper place at the foot of the page, it is more satisfactory to have the original twenty-year-old text rather than a partially revised one. By the nature of things, a considerable and pioneering thesis is not revisable. The original edition was produced at a time when interest was beginning to be taken in the history of scientific instruments, and it helped to quicken that interest, stressing as it does the social and economic factors as well as the industrial and technical ones. As a result, much new knowledge has been acquired and new attitudes have developed, but little of it has yet been published in book form. Now is the time for a completely new assessment, which, if it were to achieve the same scope as M. Maurice Daumas's work, would fill several volumes. Today, one would certainly criticize M. Daumas for deliberately omitting sundial and globe makers. There is also the curious omission of any reference to the many pre-war papers of von Rohr on the history of optical instruments.

In this English edition, one would have hoped for better sub-editing. The bibliography, now treble the original seventy items, does not contain all the works cited in the references, and, moreover, it has been only haphazardly brought up to date. Two important bibliographies on the history of astronomical and optical instruments, published in 1963 and 1969, are not included. Cross-referencing among the end notes can infuriatingly break down. For no good reason the two-page appendix is printed in French. The caption to Fig. 71 is a translation of the original caption, but the plate has been changed, so making nonsense. Fig. 141 is not of an Atwood fall-machine, but of an apparatus somewhat similar in appearance for the study of friction on an axle.

It may be that the publisher intended this book for collectors and the antiques trade, but the illustrations are not selected for this market, and the text certainly is not meant for it. It is difficult to imagine what useful purpose this English edition can serve, particularly at so high a price.

G. L'E. TURNER

Erratum

WE apologize for an unfortunate error in the review "Planetary Exploration" (*Nature*, **238**, 295; 1972), the book *Geological Problems in Lunar and Planetary Research* (AAS Publications: Tarzana, California, 1971) is not free but costs \$19.75.

CORRESPONDENCE

Avery in Retrospect

SIR,—Dr H. V. Wyatt has drawn attention to the muted manner in which Avery, MacLeod and McCarty expressed themselves in 1944 on the significance for genetics of their work on the transforming principle of pneumococcus¹. He has exposed the tendency of later commentators to read more "knowledge" into the statements of "information" than is perhaps justified. At the same time it is only fair to the three Rockefeller scientists to state Avery's reasons for the narrow delimitation of this work and for the non-committal discussion of its significance. These features can be highlighted by a comparison of the 1944 paper with papers written by André Boivin and his collaborators who worked first at the Pasteur Institute then at the University of Strasbourg.

In May 1943 O. T. Avery wrote to his brother Roy a famous letter from which Dr Wyatt quotes the phrase "Sounds like a virus—may be a gene". Avery then added, as if hastily: "But with mechanisms I am not now concerned. One step at a time and the first step is, what is the chemical nature of the transforming principle? Someone else can work out the rest. Of course, the problem bristles with implications"². He went on to assure his brother that a lot of well documented evidence was needed before anyone could be convinced that protein-free DNA had the properties he claimed. This was the task he undertook. Just how the DNA acted was a separate question, the answer to which would clarify the biological significance of transformation. In this connexion it should be borne in mind that although Robinow had demonstrated nuclear structures in rod-shaped bacteria in 1942³, no case of conjugation in bacteria had been reported in 1944, and it was not until 1946⁴ that Lederberg and Tatum had good evidence of bacterial recombination. Furthermore Avery had to contend with the traditional interpretation of bacterial transformation as given by Neufeld and Levinthal in 1928⁵, Dawson in 1930⁶, Alloway in 1933⁷—that the recipient cells have retained the power to elaborate the capsular polysaccharides of several types of pneumococci and need only the stimulus of the transforming principle, this being specific for the development of only the donor type coat. The 1944 interpretation was an advance in this position.

It was the enzyme studies which formed the bulwark of Avery's case and accordingly his efforts with McCarty, after the 1944 paper, were directed at improving this evidence⁸. Here it must be conceded that trypsin and chymotrypsin alone are inadequate as agents to remove all possible types of protein from the transforming substance. Unfortunately they could not use pepsin because the DNA was damaged at the pH required for its action⁹. Here pronase, had it been known at that time, could have filled the gap. Also none of these enzymes effectively digests protein until it is denatured, hence it was possible for Mirsky to find a weak point here when he attacked the evidence from enzymology in 1947¹⁰.

In contrast to Avery, André Boivin gave all too few details of his work with Vendrely and Lehault at the Pasteur Institute on the transformation of *Escherichia coli* types. At a meeting of the Académie des Sciences in November 1945 these workers claimed to have obtained results like Avery's but using *E. coli*¹¹. In 1942, stimulated by the work of Dawson, Sia and Alloway, they had tried to effect transformation of types in *E. coli* and before seeing the 1944 paper of the Rockefeller scientists they had come to the conclusion that the transforming principle was a nucleoprotein. When they learnt of this work they removed the protein from their nucleoprotein autolysates of *E. coli* and found the nucleic acid residues still capable of transformation. At the time this work was regarded as an extension of the pneumococcus case to another bacterial species. Only later was doubt thrown on Boivin's work after subsequent attempts to reproduce it failed¹².

To the historian it is of interest that Boivin was prepared to go much further than Avery in his interpretation of the work. The title of his paper in *Experientia* contains the phrase "Significance for the Biochemistry of Heredity". In the conclusion the discovery of the identity of the transforming principle is described as opening "new horizons", promising for the biochemistry of heredity. "In particular, it is on the side of the nucleic acid and not at all on that of the protein of the nucleoprotein macromolecule constituting a gene that one must find the basis for the inductive properties belonging to the gene"¹³. When Boivin attended the Cold Spring Harbor Symposium on "Nucleic Acids and Nucleoproteins" in June 1947, he

gave a remarkable paper¹⁴ in which he related the work on bacterial transformation to Beadle and Tatum's work on biochemical genetics, described Tulasne's confirmation of Robinow's work on the bacterial nucleus¹⁵, and the chemical mechanism involved (Vendrely and Lipardy¹⁶), and gave Tulasne's and Vendrely's cytochemical evidence, using RNase and DNase, for the localization of RNA in the bacterial cytoplasm and DNA in the nucleus of *E. coli*¹⁷.

When we look back over the mass of literature in the 1940s it seems scarcely possible that André Boivin could have so accurately predicted the structure which the nascent subject of molecular genetics was to take. Consider the following statement¹⁴: "We may, at the most, catch a glimpse of a series of catalytic actions which set out from primary directing centres (the deoxyribonucleic genes) proceed through secondary directing centres (the ribonucleic microsomes-plasma-genes) and thence through tertiary directing centres (the enzymes), to determine finally the nature of the metabolic chains involved, and to condition by this very means, all the characters of the cell in consideration . . .".

Although the Avery, MacLeod and McCarty paper was published in a journal with a fairly limited readership, the subsequent papers in New York, Atlantic City, Hershey (Pennsylvania), and Cold Spring Harbor in 1946, and in the latter again in 1947, brought bacterial transformation to the attention of a wide audience. In addition Luria, Dobzhansky and Burnet visited Avery personally in the 1940s. In war-torn Europe conditions were not conducive to the public discussion of Avery's work, yet in Paris André Lwoff and Boris Ephrussi held a colloquium with support from the Rockefeller Foundation at which the new work which had had its genesis in Avery's discovery was reported.

This new work concerned the demonstration that bacterial transformation was not confined to one hereditary characteristic and that DNA did have the properties required of the hereditary substance. When we see Hotchkiss's and Chargaff's evidence against the tetranucleotide hypothesis, Chargaff's demonstration of the species specific base composition of DNA, and the Boivin Vendrely Rule governing the DNA content of diploid and haploid cells as the fruit of work initiated by the

Avery, MacLeod, McCarty discovery, it is no longer possible to maintain that their paper was either ignored or unknown. When we see how little was known about genetic processes in bacteria and the chemistry of DNA in 1944 compared with 1950, Avery's caution can be seen as justified. As for the geneticists, it is clear that what caught their imagination was not the identity of the transforming principle—whether it was nucleic acid or nucleoprotein did not mean a great deal to them—but the possibility, at last, to bring about a given hereditary change by a specific treatment. Hence the reason for the widespread habit of referring to bacterial transformation as "directed mutation".

H. J. Muller was exceptional among geneticists in being concerned about the chemical identity of Avery's transforming principle, but was impressed by Mirsky's opinion. To Darlington he wrote: "... Mirsky gave reasons for believing that Avery's so-called nucleic acid is probably nucleoprotein after all ...".¹⁸ Yet again, what attracted Muller was the possibility of fitting transformation into the grand scheme of cytogenetics. In the transforming substance, he suggested, there were chromosomal fragments consisting of nucleoprotein, which were incorporated into the genetic apparatus of the recipient bacterium in transformation.

As in the case of Mendel's paper, the scientists of a given period found in Avery's paper what they were looking for, but unlike that earlier case, the 1944 paper was not ignored or unknown. It posed questions about DNA which by 1950 could be answered. What Avery failed to say, Boivin said, but his brave words did not profoundly alter the climate of opinion until the Boivin Vendrely Rule was established¹⁹. Only with the advantage of hindsight can we see the significance of the 1944 paper as obvious. Only by confining our

attention to the published record and the citation statistics on Avery's paper can we arrive at the view that it was little known or undervalued.

Yours faithfully,

R. OLBY

Department of Philosophy,
University of Leeds,
Leeds LS2 9JT

- ¹ Wyatt, H. V., *Nature*, **235**, 86 (1972).
- ² Hotchkiss, R. D., in *Phage and the Origins of Molecular Biology* (edit. by Cairns, J., Stent, G. S., and Watson, J. D.) (Cold Spring Harbor Laboratory, 1966).
- ³ Robinow, C. F., *Proc. Roy. Soc., B*, **130**, 299 (1942).
- ⁴ Lederberg, J., and Tatum, E. L., *Nature*, **158**, 558 (1946).
- ⁵ Neufeld, F., and Levinthal, W., *Z. Immunitätsforsch. Exp. Ther.*, **55**, 324 (1928).
- ⁶ Dawson, M. H., *J. Exp. Med.*, **51**, 143 (1930).
- ⁷ Alloway, J. L., *J. Exp. Med.*, **57**, 265 (1933).
- ⁸ McCarty, M., and Avery, O. T., *J. Exp. Med.*, **83**, 89 (1946).
- ⁹ Avery, O. T., MacLeod, C., and McCarty, M., *J. Exp. Med.*, **79**, 137 (1944).
- ¹⁰ Mirsky, A. E., *Cold Spring Harbor Symp. Quant. Biol.*, **12**, 16 (1947).
- ¹¹ Boivin, A., Vendrely, R., and Lehault, Y., *C.R. Acad. Sci., Paris*, **221**, 646 (1945).
- ¹² Avadhani, N.-G., Mehta, B. M., and Rege, D. V., *J. Mol. Biol.*, **42**, 413 (1969).
- ¹³ Boivin, A., Delaunay, A., Vendrely, R., and Lehault, Y., *Experientia*, **1**, 334 (1945).
- ¹⁴ Boivin, A., *Cold Spring Harbor Symp. Quant. Biol.*, **12**, 7 (1947).
- ¹⁵ Tulasne, R., *CR Seanc. Soc. Biol.*, **141**, 411 (1947).
- ¹⁶ Vendrely, R., and Lipardy, J., *C.R. Acad. Sci., Paris*, **223**, 342 (1946).
- ¹⁷ Tulasne, R., and Vendrely, R., *C.R. Seanc. Soc. Biol.*, **141**, 674 (1947).
- ¹⁸ Letter from H. J. Muller to C. D. Darlington dated March 2, 1946.
- ¹⁹ Boivin, A., Vendrely, R., and Vendrely, C., *C.R. Acad. Sci.*, **226**, 1061 (1948).

Peregrines and Propaganda

SIR,—Dr Cramp writes that "there is more than propaganda to justify the

belief that the persistent pesticides led to striking declines in peregrines" (*Nature*, **238**, 475; 1972). He then refers to evidence, as if the existence of evidence sufficed to make the case, irrespective of contrary evidence or unsoundness. But rarely is there absolutely no evidence behind propaganda. In this case, the evidence was examined by the Wilson Committee¹ and the Mrak Commission² and found to be inadequate.

Dr Cramps chides me for not quoting the Wilson Report for the suggestion that dieldrin was responsible; but that was just a suggestion and not altogether convincing. In this country, for most purposes the small tonnages of dieldrin and DDT used could be replaced by other insecticides, as recommended by the Wilson Committee, because there were few disadvantages in doing so. But the balance of advantage would be quite different in some countries, where the lives and happiness of many millions of human beings would be put at risk by abandoning DDT and dieldrin. In those countries, much more rigorous examination of and search for evidence would be essential and mere suggestions ought not to be lightly accepted. In particular, in this country and in North America, suggestions and insufficient evidence are converted by propaganda into beliefs³; such beliefs will be accepted by malarious countries at their peril.

Yours faithfully,

D. L. GUNN

Chilham,
Kent

¹Advisory Committee on Pesticides and other Toxic Chemicals, 148 (HMSO, 1969).

²Report of the Secretary's Commission on Pesticides and their Relationship to Environmental Health, 677 (US Department of Health, Education and Welfare, 1969).

³Gunn, D. L., *Ann. Appl. Biol.*, **72** (in the press).

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